

## Research Article

# Physical and Physicochemical Characteristics, Bioactive Compounds, and Antioxidant Activity of Cladodes from Erect Prickly Pear *Opuntia stricta* (Haw.) Haw.

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*Opuntia stricta* (Haw.) Haw. is well adapted to arid and semiarid regions and has great potential for industrialisation. One- to four-year-old cladodes of this species harvested in Drâa-Tafilalet region in the southeast of Morocco, were characterised by examining physical and physicochemical parameters. Their bioactive compounds and antioxidant activity were also investigated. The cladode age had a significant impact on all the studied characteristics except some morphological parameters. Cladodes of different ages have a nutritional value comparable to a wide range of vegetables, particularly in terms of calcium, potassium, betalain, total polyphenol, and vitamin C contents. They are very rich in calcium (37.8–42.5 g/kg of dry matter (DM)) and have high betalain content. The content of phenolic compounds (133.9–165.0 mg gallic acid equivalent (GAE)/100 g DM) is especially noteworthy. These cladodes also contain very high amounts of vitamin C, especially the one-year-old cladodes (8.1–18.5 mg GAE/100 g DM). The recorded flavonoid contents vary from 3.8 to 7.6 mg of quercetin equivalent per 100 mL of cladodes mash. Condensed tannin contents range from 10.9 to 21.8 µg/100 mL of cladodes mash. Chlorophyll and carotenoid contents also vary from 5.55 to 23.76 and from 1.9 to 6.0 mg/100 g DM, respectively. One- and two-year-old cladodes are rich in total soluble solids (TSS) and ash with high total titratable acidity (TTA), compared to three- and four-year-old cladodes. Four-year-old cladodes have the highest TSS/TTA ratio, while two-year-old cladodes are the wealthiest in betalains with a dominance of indicaxanthins (yellow pigments). The oldest cladodes have the lowest inhibitory concentration (IC<sub>50</sub>) for antioxidant activity. Three-year-old cladodes have some traits significantly different, which may have resulted from physiological changes related to their great buds emission at this age. This study highlights the nutritional and functional potential of *Opuntia stricta* cladodes and the importance of their valorisation, particularly in agri-food and pharmaceutical industries.

## 1. Introduction

One of the biggest problems the world is currently experiencing is climate change [1]. This phenomenon is most pronounced in arid and semiarid areas where the climate is becoming substantially drier and the rainfall is increasingly

low, erratic, and highly unpredictable. Rapid population growth, overgrazing, and poor soil fertility are additional problems that are hastening the process of desertification, especially in drylands [2]. The prickly pear (also named cactus) is receiving increasing attention across the globe because of its physiological characteristics, which enable it to

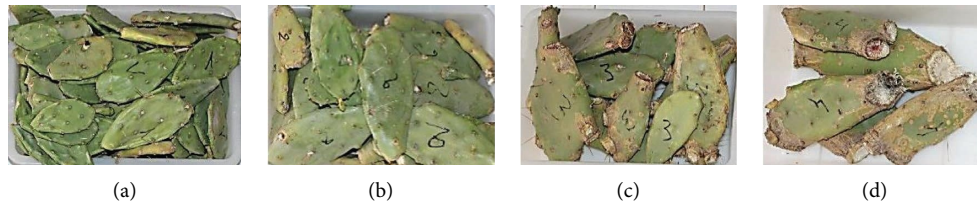


FIGURE 1: Cladodes of *Opuntia stricta* (erect prickly pear) at four ages. (a) 1-year-old cladodes; (b) 2-year-old cladodes; (c) 3-year-old cladodes; (d) 4-year-old cladodes.

grow and survive in very adverse weather conditions and on poor substrates, in coastal and continental areas [2, 3].

Compared to other cacti, *Opuntia* species have a higher commercial and ecological interest [4]. They are cultivated for fruit and forage production, and therefore used as a tool to mitigate drought and combat desertification [5, 6]. They are also used as a source of products for subsistence farming, contributing to the food security of populations in marginal lands. Additionally, fruits and cladodes are very rich in terms of nutritional and bioactive compounds, which give them a wide scope of application in the agri-food and cosmetic industries, and in traditional medicine to prevent and/or treat infections and chronic diseases [7–9].

Several scientific studies on the physical, chemical, biochemical characterisation, and identification of bioactive compounds in *Opuntia* cladodes have already been carried out [8–12]. Cladodes are also employed to extract juice, powder, and bioactive compounds which are used for the formulation of a wide range of food products or functional foods [13]. The cladode age could have a large effect on quality parameters. For this, the current study examines the effect of four different ages (1, 2, 3, and 4 years old) on the physical and physicochemical characteristics, bioactive compounds, and antioxidant activity of *Opuntia stricta* (Haw.) Haw. cladodes.

## 2. Materials and Methods

**2.1. Plant Material.** The cladodes of *Opuntia stricta* (Haw.) Haw. at four different ages (1, 2, 3, and 4 years old) (Figure 1) were harvested from the cactus germplasm maintained at the INRA Experimental Domain in Errachidia, Drâa-Tafilalet region in the southeast of Morocco (31°55'7.00"N; 4°26'59.2"W; 1043 m above sea level (a.s.l.)). They had previously been collected from Erfoud in the same region (31°25'08.9"N; 4°17'16.7"W; 803 m a.s.l.). They were sorted and cleaned to remove dust and any impurities. Forceps were used to delicately remove the cladode spines. Cladodes were then manually divided into approximately 1 cm<sup>2</sup> sized pieces using a knife. Afterwards, they were ground at a speed of 4000 rpm for 80 s in a knife mill mixer (GM 200, Grindomix-Retsch, Germany). The resulting mash was packed in airtight boxes, labelled, and stored in a freezer at –20°C until analysis.

**2.2. Determination of Morphological Parameters.** For each age, morphological parameters were determined on freshly harvested cladodes: colour, shape, weight (g), length (cm), bottom width (cm), middle width (cm), top width (cm), bottom thickness (cm), middle thickness (cm), top thickness

(cm), number of areolas per face, number of buds, and distance between areolas (cm) (Figure 2). Morphological measurements were performed on five cladodes of each age.

**2.3. Colour Determination.** The colour of the mashed cladodes was measured with a colorimeter for solids (Techkon, SpectroDens, Germany) using the CIELAB system which expresses colour as three values  $L^*$ ,  $a^*$ , and  $b^*$ . The value of the lightness  $L^*$  varies from 0 (black) to 100 (white) and the two parameters  $a^*$  and  $b^*$  express the deviation of the colour regarding a grey surface of the same lightness. The value of  $a^*$  represents the red/green coordinate with a range of 200 levels on the red axis (+100) to green (–100) through grey (0). The value of  $b^*$  represents the yellow/blue coordinate with a range of 200 levels on the yellow axis (+100) to blue (–100) through grey (0). The three colour parameters were measured by performing 10 repetitions for all ages. The polar coordinates  $C_{ab}^*$  and  $h_{ab}$  in the plane ( $a^*$ ,  $b^*$ ) were also calculated to represent the chromaticity (or the chroma) and the chrominance (or the hue angle) using equations (1) and (2), respectively:

$$C_{ab}^* = \sqrt{a^{*2} + b^{*2}}, \quad (1)$$

$$h_{ab} = \arctan \frac{b^*}{a^*}. \quad (2)$$

**2.4. Moisture Analysis.** The moisture content of cladodes of the four ages (expressed in g/100 g of fresh matter (FM)) was determined by drying a sample of 5 g of cladodes mash in an oven (Function Line, Germany) at atmospheric pressure and a temperature of 103 ± 2°C for 48 hours [14]. Three replicates were performed.

**2.5. Total Soluble Solids (TSS) Analysis.** The TSS (expressed in %) of cladodes mash was measured by refractometry. The reading of the TSS is made at the standard temperature of 20°C with a digital refractometer (RFM 830, UK). Three replicates were performed.

**2.6. Total Titratable Acidity (TTA) Analysis.** To determine the TTA of cladodes, the extract to be titrated was prepared from a quantity varying from 50 to 70 g of cladodes mash. These quantities were added to distilled, boiled, and neutralised water. The samples were boiled for 30 min while being stirred. After filtration, the filtrate was titrated by adding NaOH (0.1 N) solution to pH = 8.1 [14]. Results are

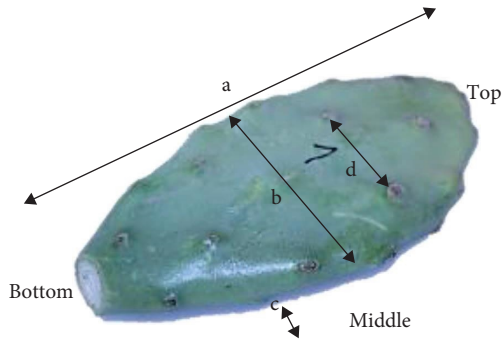


FIGURE 2: Morphological parameters studied for *Opuntia stricta* cladodes: (a) length; (b) width; (c) thickness; (d) distance between areolas.

expressed in g citric acid/100 g FM. Three replicates were performed.

**2.7. pH Analysis.** The pH was measured directly in 30 g of cladodes mash at 25°C using a pH meter. Three replicates were performed.

**2.8. Ash and Minerals Analysis.** To determine the ash content (expressed in g/100 g DM), 5 g of the cladodes mash were placed for incineration for 7 hours at 500°C in a muffle furnace (Thermolyne type 1400, USA). Three replicates were performed. For determining the contents of sodium (Na), calcium (Ca), potassium (K), and barium (Ba), the ash samples were solubilised with nitric acid, filtered through ash-free filter paper, and then put into 100 mL flasks that had been filled with demineralised water up to the mark. The analysis of minerals (expressed in g/kg DM) was performed by a flame spectrophotometer (BWB, UK).

**2.9. Total Polyphenols and Ascorbic Acid Analysis.** A mixture was obtained by mixing 10 g of the mashed cladodes with 10 mL of pure acetone. This mixture was then centrifuged at 5000 rpm for 15 min and filtered using 90 mm filter paper. The extract obtained was used for the determination of total polyphenols and vitamin C contents (expressed in mg gallic acid equivalent (GAE)/100 g DM) according to the Folin–Ciocalteu method [15]. The optical density was measured at 760 nm using a Ultraviolet-Visible (UV-Vis) spectrophotometer (Varian Cary 50 Conc, USA).

**2.10. Flavonoids Analysis.** The extraction of flavonoids contained in cladodes was performed according to the protocol described by Mahmoudi et al. [16]. The

determination of total flavonoids was performed by the method of Dehpour et al. [17]. The optical density was measured at 415 nm using a UV-Vis spectrophotometer (Varian Cary 50 Conc, USA). Results are expressed in mg quercetin equivalent (QE)/100 mL of cladodes mash.

**2.11. Condensed Tannins Analysis.** The aqueous extraction described by Mahmoudi et al. [16] was used to extract condensed tannins from cladodes mash. The dosage of these components was performed according to the method described by Ba et al. [18]. The absorbance was measured at 500 nm using a UV-Vis spectrophotometer (Varian Cary 50 Conc, USA). Results are expressed in µg catechin equivalent (CE)/100 mL of cladodes mash.

**2.12. Betalains Analysis.** The analysis of betalains with their two types: betacyanins and indicaxanthins, was performed in cladodes mash according to the method described by Chougui et al. [19]. Each analysis was performed in three replicates. To quantify the content of betacyanins and indicaxanthins, expressed in mg/100 g DM, the molecular weight (MW) and molar extinction coefficient ( $\xi$ ) of betacyanins (MW = 550 g/mol;  $\xi$  = 60.000 L/mol × cm;  $\lambda$  = 538 nm) and those of indicaxanthins (MW = 308 g/mol;  $\xi$  = 48.000 L/mol × cm;  $\lambda$  = 480 nm) were applied according to the equation (3) (A, DF, V, l, W, and DM are as follows: absorbance value measured with UV-Vis spectrophotometer (Varian Cary 50 Conc, USA), dilution factor, volume of extraction solvent (mL), path length of the cuvette (1 cm), sample weight (g), and dry matter (g/100 g), respectively):

$$\text{Betalain content} \left( \frac{\text{mg}}{100 \text{ g DM}} \right) = \frac{(A \times DF \times MW \times V \times 10000)}{(\xi \times l \times W \times DM)} \quad (3)$$

**2.13. Photosynthetic Pigments Analysis.** The analysis of photosynthetic pigments, especially chlorophylls a and b and carotenoids was performed in cladodes mash according to the method of Lichtenthaler [20]. Their optical densities (OD) were read at wavelengths  $\lambda_a$  = 647 nm,  $\lambda_b$  = 663 nm, and  $\lambda_c$  = 470 nm, respectively using a UV-Vis spectrophotometer (Varian Cary 50 Conc, USA). The results are expressed in mg/100 g DM according to the equations (4)–(7) (Xan, Car1, Car2, Chla, Chlb, V, W, and DM are xanthophylls, carotenes, carotenoids, chlorophyll a, chlorophyll b, volume of extraction solvent (mL), sample weight (g), and dry matter (g/100 g), respectively):

$$\text{Chla} \left( \frac{\text{mg}}{100 \text{ g DM}} \right) = \frac{(12.25 \times \text{OD}_{\lambda_b} - 2.79 \times \text{OD}_{\lambda_a}) \times V \times 10}{(W \times \text{DM})} \quad (4)$$

$$\text{Chlb} \left( \frac{\text{mg}}{100 \text{ g DM}} \right) = \frac{(21.5 \times \text{OD}_{\lambda_a} - 5.1 \times \text{OD}_{\lambda_b}) \times V \times 10}{(W \times \text{DM})} \quad (5)$$

TABLE 1: Morphological parameters of *Opuntia stricta* fresh cladodes at four ages (\*).

Parameters	Cladode ages (years)			
	1	2	3	4
Weight (g)	139.10 ± 39.46 <sup>a</sup>	231.28 ± 33.36 <sup>a</sup>	402.21 ± 143.03 <sup>b</sup>	408.39 ± 57.05 <sup>b</sup>
Length (cm)	20.20 ± 0.76 <sup>a</sup>	21.70 ± 1.25 <sup>a</sup>	25.38 ± 3.33 <sup>a</sup>	22.58 ± 4.05 <sup>a</sup>
Bottom width (cm)	3.04 ± 0.30 <sup>a</sup>	3.42 ± 0.26 <sup>a</sup>	3.98 ± 0.74 <sup>ab</sup>	4.94 ± 0.55 <sup>b</sup>
Middle width (cm)	7.90 ± 0.80 <sup>a</sup>	9.17 ± 0.87 <sup>a</sup>	9.86 ± 1.74 <sup>a</sup>	8.95 ± 0.77 <sup>a</sup>
Top width (cm)	3.52 ± 0.18 <sup>a</sup>	3.98 ± 0.72 <sup>a</sup>	4.26 ± 0.88 <sup>a</sup>	5.14 ± 1.54 <sup>a</sup>
Bottom thickness (cm)	1.66 ± 0.39 <sup>a</sup>	2.56 ± 0.32 <sup>ab</sup>	3.20 ± 0.87 <sup>b</sup>	3.74 ± 0.40 <sup>c</sup>
Middle thickness (cm)	1.32 ± 0.45 <sup>a</sup>	1.48 ± 0.26 <sup>a</sup>	1.78 ± 0.35 <sup>a</sup>	2.30 ± 1.03 <sup>a</sup>
Top thickness (cm)	1.06 ± 0.30 <sup>a</sup>	1.72 ± 0.28 <sup>b</sup>	2.12 ± 0.11 <sup>b</sup>	2.86 ± 0.47 <sup>c</sup>
Number of buds	0.00 ± 0.00 <sup>a</sup>	0.60 ± 0.89 <sup>a</sup>	4.20 ± 2.17 <sup>b</sup>	1.80 ± 1.79 <sup>ab</sup>
Number of areolas per face 1	18.20 ± 1.10 <sup>a</sup>	16.40 ± 2.19 <sup>a</sup>	17.40 ± 3.13 <sup>a</sup>	18.00 ± 3.32 <sup>a</sup>
Number of areolas per face 2	18.20 ± 2.77 <sup>a</sup>	17.80 ± 1.10 <sup>a</sup>	16.00 ± 4.06 <sup>a</sup>	14.20 ± 3.11 <sup>a</sup>
Distance between areolas in bottom (cm)	2.42 ± 0.40 <sup>a</sup>	2.98 ± 0.57 <sup>ab</sup>	3.80 ± 1.04 <sup>b</sup>	3.60 ± 0.42 <sup>ab</sup>
Distance between areolas in middle (cm)	4.22 ± 1.34 <sup>a</sup>	4.28 ± 0.72 <sup>a</sup>	5.52 ± 0.53 <sup>a</sup>	5.20 ± 1.48 <sup>a</sup>
Distance between areolas in top (cm)	3.04 ± 0.59 <sup>a</sup>	3.74 ± 0.49 <sup>ab</sup>	4.20 ± 0.27 <sup>ab</sup>	4.96 ± 1.24 <sup>b</sup>

(\*) For each parameter, means ± standard deviations with the same letter do not differ significantly according to the Scheffé test at  $P < 0.05$ .

$$\text{Chla} + \text{Chlb} \left( \frac{\text{mg}}{100 \text{ g DM}} \right) = \frac{(7.15 \times \text{OD}\lambda b + 18.71 \times \text{OD}\lambda a) \times V \times 10}{(W \times \text{DM})}, \quad (6)$$

$$\text{Car2} (\text{Xan} + \text{Car1}) \left( \frac{\text{mg}}{100 \text{ g DM}} \right) = \frac{(1000 \times \text{DO}\lambda c - 1.82 \times \text{Chla} - 85.02 \times \text{Chlb}) \times V \times 10}{(198 \times W \times \text{DM})}. \quad (7)$$

**2.14. Antioxidant Activity Determination (DPPH Test).** 10 g of cladodes mash were macerated in 10 mL of methanol/water (70/30) for 24 hours while being stirred. The extract was filtered through muslin tissue and collected in a 25 mL flask, and then the retentate was exhausted with 10 mL of methanol/water (70/30). The flask was filled to the mark with the extraction solvent. A first centrifugation was performed at 4000 rpm for 15 min at 25°C, followed by a second centrifugation at 5000 rpm for 15 min at 25°C to properly eliminate mucilaginous fibers. The free radical scavenging activity of the cladodes extract was measured by the method using DPPH described by Archana et al. [21]. The optical density was measured at a wavelength of 517 nm using a UV-Vis spectrophotometer (Varian Cary 50 Conc, USA). The percentage of inhibition was calculated using the equation (8) ( $A_s$  and  $A_c$  are the absorbance of the sample and the absorbance of the control, respectively):

$$\text{Percentage of inhibition (\%)} = \left( 1 - \left( \frac{A_s}{A_c} \right) \right) \times 100. \quad (8)$$

To determine the IC<sub>50</sub> (inhibitory concentration 50), a serial dilution was prepared from the cladode solution. The antioxidant activities were assessed, and the percentage of inhibition for each dilution was calculated. A calibration curve linking the concentrations of different dilutions and their percentage of inhibition was performed.

**2.15. Statistical Analysis of the Data.** Data are represented as mean ± standard deviation. One-way analysis of variance was used to compare the means obtained for the studied parameters of *Opuntia stricta* cladodes belonging to the four ages, followed by the Scheffé test post hoc multiple comparisons. The software used was SPSS (IBM SPSS Statistics for Windows, Version 25.0. Armonk, NY: IBM Corp.). A principal component analysis (PCA) of the data was also performed with the same software to improve the interpretability of the variability.

### 3. Results and Discussion

**3.1. Morphological Parameters.** For all studied ages, the cladode colour evaluated visually is typically green with a variety of green tones (Figure 1). Obovate and elliptical forms are the most prevalent. According to Table 1, most of the morphological parameters did not show significant differences between cladodes of different ages, except for the number of buds, weight, bottom width, bottom thickness, and distance between areolas at the top and bottom of the cladodes.

In fact, the number of buds of 3-year-old cladodes shows a strong capacity for budding. For this age, the average bud count is  $4.2 \pm 2.2$ . 3- and 4-year-old cladodes have a higher weight than 1- and 2-year-old cladodes. The bottom width and the distance between the areolas at the top of the cladodes do not show significant differences for 1-, 2-, and 3-

TABLE 2: CIELAB colour parameters of *Opuntia stricta* cladodes at four ages (\*).

Colour parameters	1-year-old cladodes	2-year-old cladodes	3-year-old cladodes	4-year-old cladodes
$L^*$	$31.46 \pm 0.30^d$	$30.17 \pm 0.73^c$	$26.37 \pm 0.39^a$	$27.74 \pm 0.50^b$
$a^*$	$-6.20 \pm 0.13^a$	$-5.16 \pm 0.35^b$	$-3.39 \pm 0.06^c$	$0.86 \pm 0.17^d$
$b^*$	$22.74 \pm 0.45^d$	$18.37 \pm 1.31^c$	$15.55 \pm 0.30^b$	$13.97 \pm 0.39^a$
$C_{ab}^*$	$23.57 \pm 0.45^d$	$19.08 \pm 1.35^c$	$15.92 \pm 0.29^b$	$13.99 \pm 0.39^a$
$h_{ab}$	$-1.31 \pm 0.01^b$	$-1.30 \pm 0.01^b$	$-1.36 \pm 0.01^a$	$1.51 \pm 0.01^c$

$L^*$ : represents lightness and varies from 0 (black) to 100 (white);  $a^*$ : represents a range on the red axis (+100) to green axis (-100) through grey (0);  $b^*$ : represents a range on the yellow axis (+100) to blue axis (-100) through grey (0);  $C_{ab}^*$ : represents chromaticity or chroma;  $h_{ab}$ : represents hue angle or chrominance. (\*) For each parameter, means + standard deviations with the same letter do not differ significantly according to the Scheffé test at  $P < 0.05$ .

year-old cladodes, except for 4-year-old cladodes, which have values of  $4.9 \pm 0.6$  cm for bottom width and  $5.0 \pm 1.2$  cm for the distance between the areolas at the top of cladodes. The bottom thickness differed significantly between cladodes of different ages with a maximum value of  $3.7 \pm 0.4$  cm recorded for 4-year-old cladodes.

**3.2. Colour.** Table 2 shows the colour parameters  $L^*$ ,  $a^*$ ,  $b^*$ , chromaticity  $C_{ab}^*$  and chrominance  $h_{ab}$  measured on the cladodes mash belonging to the four studied ages.

The analysis of variance shows that the lightness ( $L^*$ ) differs significantly between the four ages ( $P < 0.05$ ). The difference in brightness between the 1-year-old and 3-year-old cladodes is approximately 16.2%. The parameter  $a^*$  exhibits a significant difference ( $P < 0.05$ ) between cladode mashes of different ages, where the dominance of the green colour decreases with age ( $a^*$  is negative for ages from 1 to 3 years and it is positive for the age of 4 years). The  $b^*$  parameter is positive for the four ages, indicating a yellow colour shade that significantly decreases with ageing ( $P < 0.05$ ). The decrease is about 19.2%, 15.3%, and 10.2% between 1- and 2-year-old, 2- and 3-year-old, and 3- and 4-year-old cladodes, respectively. Concerning chroma values  $C_{ab}^*$ , they decrease significantly with age from  $23.57 \pm 0.45$  for 1-year-old cladodes to  $13.99 \pm 0.39$  for 4-year-old cladodes (Table 2). Chrominance  $h_{ab}$  is almost the same for 1- and 2-year-old cladodes ( $P > 0.05$ ), whereas it differs significantly in cladodes of other ages. These variations of colour parameters ( $L^*$ ,  $a^*$ ,  $b^*$ ,  $C_{ab}^*$  and  $h_{ab}$ ) according to cladode ages indicate a disappearance of green colour in favour of yellow colour and a decrease in lightness. This might be due to the degradation of chlorophyll pigments in the older cladodes. Indeed, the progressive loss in green colour with age can be explained by the variation of photosynthetic pigments equilibrium in cladodes, which is mainly influenced by the degradation of chlorophyll pigments as the cladodes senesce.

**3.3. Moisture.** The moisture contents in cladodes were  $84.29 \pm 0.01\%$ ,  $85.75 \pm 0.03\%$ ,  $85.15 \pm 0.04\%$ , and  $85.90 \pm 0.07\%$  for 1-, 2-, 3-, and 4-year-old cladodes, respectively. Variance analysis showed a significant difference between the different ages of cladodes ( $P < 0.05$ ) (Table 3). Relatively high moisture values of erect prickly pear cladodes explain well their perishability, rapid spoilage, and limited storage ability. They are directly related to the typical crassulacean acid metabolism of cacti. These moisture values are lower than those reported by Stintzing and Carle [22] for *Opuntia* spp. cladodes (varying between 88% and 95%) and by Harrak [23] for cladodes of

*Opuntia ficus-indica* species aged less than one-year-old and up to 3.5 years old (varying between 89.77% and 92.61%). This fact can be explained by the arid environment where the cladodes were grown (Drâa-Tafilalet region).

**3.4. Total Soluble Solids.** An inverse relationship between TSS and cladode age was observed. The TSS percentage decreases significantly with cladode age:  $10.1 \pm 0.1\%$ ,  $10.0 \pm 0.2\%$ ,  $9.3 \pm 0.1\%$ , and  $8.0 \pm 0.1\%$  for 1-, 2-, 3-, and 4-year-old cladodes, respectively (Table 3). The TSS values found in this work are comparable to that found by Gómez-López et al. [24] for the fruit of *Opuntia stricta* var. *dillenii* ( $10.80 \pm 0.30\%$ ). These *Opuntia stricta* TSS values are significantly higher than those found by Harrak [23] for *Opuntia ficus-indica* cladodes of four different ages varying between 5.8% and 4.1% and that reported by Legrine et al. [8] ( $5.5 \pm 0.5\%$ ) for *Opuntia ficus-indica*. This could be explained by the aridity of the environment where the studied cladodes were collected (Drâa-Tafilalet region). Indeed, fruits and vegetables grown in dry areas have a sweeter flavour than those grown in wet areas [25] or irrigated areas [26].

**3.5. pH.** Significant differences ( $P < 0.05$ ) were recorded between pH cladodes mash at the four studied ages. The pH values are:  $4.32 \pm 0.01$ ,  $4.55 \pm 0.02$ ,  $4.44 \pm 0.02$ , and  $4.62 \pm 0.01$  for 1-, 2-, 3-, and 4-year-old cladodes, respectively (Table 3). This acidic pH of *Opuntia stricta* cladodes can be explained by the acidic metabolism of this plant. It is remarkably similar to that found by Harrak [23] (ranging between 4.2 and 4.4) for cladodes of four different ages of *Opuntia ficus-indica*. This pH is also consistent with that found by Boutakiout [10] in the juice of *Opuntia ficus-indica* and *Opuntia megacantha* cladodes (ranging between 4.5 and 4.8) and that reported by Kamal et al. [8] for *Opuntia ficus-indica* cladodes ( $4.57 \pm 0.02$ ).

**3.6. Total Titratable Acidity.** The TTA, which is a very good indicator of the intensity of acidic flavour [27], shows significant differences between cladodes of the four ages. This acidity is inversely proportional to cladode age. Indeed, the highest TTA was recorded for 1-year-old cladodes, with a value of  $0.493 \pm 0.003$  g citric acid/100 g FM. The lowest TTA was recorded for 4-year-old cladodes with a value of  $0.251 \pm 0.001$  g citric acid/100 g FM (Table 3). These TTA values are higher than those found by Harrak [23], ranging from 0.11 to 0.29 g citric acid/100 g FM for *Opuntia ficus-*

TABLE 3: Physicochemical parameters of *Opuntia stricta* cladodes at four ages (\*).

Age (years)	Moisture (g/100 g FM)	TSS (%)	pH	TTA (g CA/100 g FM)	TSS/TTA ratio	Ash (g/100 g DM)	Potassium (g/kg DM)	Calcium (g/kg DM)	Sodium (g/kg DM)	Barium (g/kg DM)
1	84.29 ± 0.01 <sup>a</sup>	10.1 ± 0.1 <sup>c</sup>	4.32 ± 0.01 <sup>a</sup>	0.493 ± 0.0003 <sup>c</sup>	20.55 ± 0.11 <sup>b</sup>	34.38 ± 0.02 <sup>c</sup>	6.48 ± 0.78 <sup>ab</sup>	39.09 ± 1.50 <sup>ab</sup>	0.00 ± 0.00 <sup>a</sup>	19.18 ± 0.80 <sup>a</sup>
2	85.75 ± 0.03 <sup>c</sup>	10.0 ± 0.2 <sup>c</sup>	4.55 ± 0.02 <sup>c</sup>	0.510 ± 0.001 <sup>d</sup>	19.69 ± 0.25 <sup>a</sup>	36.37 ± 0.12 <sup>d</sup>	8.10 ± 1.12 <sup>ab</sup>	42.52 ± 1.43 <sup>b</sup>	0.00 ± 0.00 <sup>a</sup>	21.28 ± 0.32 <sup>b</sup>
3	85.15 ± 0.04 <sup>b</sup>	9.3 ± 0.1 <sup>b</sup>	4.44 ± 0.02 <sup>b</sup>	0.438 ± 0.002 <sup>b</sup>	21.33 ± 0.13 <sup>c</sup>	32.03 ± 0.39 <sup>b</sup>	8.86 ± 0.71 <sup>b</sup>	41.33 ± 0.51 <sup>ab</sup>	0.00 ± 0.00 <sup>a</sup>	21.24 ± 1.07 <sup>b</sup>
4	85.90 ± 0.07 <sup>d</sup>	8.0 ± 0.1 <sup>a</sup>	4.62 ± 0.01 <sup>d</sup>	0.251 ± 0.001 <sup>a</sup>	31.92 ± 0.37 <sup>d</sup>	30.63 ± 0.26 <sup>a</sup>	4.57 ± 0.40 <sup>a</sup>	37.78 ± 0.15 <sup>a</sup>	0.00 ± 0.00 <sup>a</sup>	19.97 ± 0.10 <sup>ab</sup>

(\*) Means ± standard deviations with the same letter within the column do not differ significantly according to Scheffé test at  $P < 0.05$ . TSS: total soluble solids; TTA: total titratable acidity; CA: citric acid; FM: fresh matter; DM: dry matter.

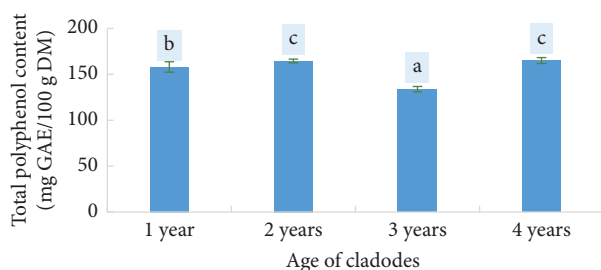


FIGURE 3: Total polyphenol content of *Opuntia stricta* cladodes at four ages (GAE: gallic acid equivalent; DM: dry matter; means with the same letter do not differ significantly according to Scheffé test at  $P < 0.05$ ; error bars represent the standard deviation of means).

*indica* cladodes. Andreu et al. [28] found values ranging from  $2.56 \pm 0.09$  to  $5.05 \pm 0.11$  g citric acid/L of *Opuntia ficus-indica* cladode juice. The TTA of *Opuntia stricta* cladodes is comparable to other fruit juices but lower than lemon and orange juices because of their richness in citric acid:  $0.94 \pm 0.02$  g citric acid/kg for pink pomelo juice (*Citrus grandis*) [29],  $2.72 \pm 0.08$  g citric acid/L for pomegranate juice (*Punica granatum*) [30],  $7.8 \pm 0.30$  g citric acid/kg for orange juice [31], and  $52.40 \pm 2.50$  g citric acid/L for lemon juice (*Citrus limon*) [32].

**3.7. Total Soluble Solids/Total Titratable Acidity Ratio.** Flavour is generally related to the relative proportions of sugars and acids in fruits and vegetables. The TSS/TTA ratio is a very good indicator to judge the quality of these products tastes. 4-year-old cladodes had the highest TSS/TTA ratio, which was  $31.92 \pm 0.37$ , followed by 3-, 1-, and 2-year-old cladodes with values of  $21.33 \pm 0.13$ ,  $20.55 \pm 0.11$ , and  $19.69 \pm 0.25$ , respectively ( $P < 0.05$ ). According to these ratios, it can be concluded that cladodes of the four ages differed in taste, especially 4-year-old cladodes (Table 3).

**3.8. Ash and Minerals.** A significant variation ( $P < 0.05$ ) is recorded between cladodes of the four ages regarding the ash content, which provides information on the richness of minerals in a plant (Table 3). The highest content was recorded for 1-year-old cladodes ( $36.37 \pm 0.12$  g/100 g DM), followed by 2-year-old cladodes ( $34.38 \pm 0.02$  g/100 g DM). The 4-year-old cladodes are the poorest in ash, with a value of  $30.63 \pm 0.26$  g/100 g DM. However, the recorded ash contents in *Opuntia stricta* cladodes are very high. This richness can be explained by the presence of crystalline structures formed by calcium oxalate on the majority of epidermal cells and some parenchymal cells [33]. The content found in this work is significantly higher than that found by Astello-García et al. [11] for different species: 14.4% for *Opuntia ficus-indica*, 13.2% for *Opuntia albicarpa*, 13.6% for *Opuntia megacantha*, 15.1% for *Opuntia hyptiacantha* and 12.6% for *Opuntia streptacantha*. They are also higher than the ash contents found by Hadj Sadok [34] ranging from 12% to 15% for *Opuntia ficus-indica*. By comparing to other fruits, the ash contents of *Opuntia stricta* cladodes of different ages are much higher than those recorded for ten Moroccan cultivars of pomegranate varying

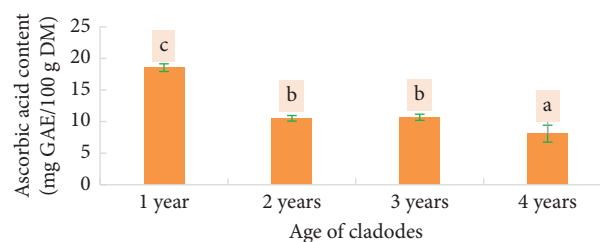


FIGURE 4: Ascorbic acid content of *Opuntia stricta* cladodes at four ages (GAE: gallic acid equivalent; DM: dry matter; means with the same letter do not differ significantly according to Scheffé test at  $P < 0.05$ ; error bars represent the standard deviation of means).

from 0.19 to 0.39 g/100 g FM (corresponding to 1.27 and 2.91 g/100 g DM, respectively) [35, 36].

Regarding the studied minerals (Table 3), the most abundant mineral for cladodes of the four ages is Ca with  $39.09 \pm 1.50$ ,  $42.52 \pm 1.43$ ,  $41.33 \pm 0.51$ , and  $37.78 \pm 0.15$  g/kg DM recorded for 1-, 2-, 3-, and 4-year-old cladodes, respectively. Ba was the second most abundant mineral with  $19.18 \pm 0.80$ ,  $21.28 \pm 0.32$ ,  $21.24 \pm 1.07$ , and  $19.97 \pm 0.10$  g/kg DM recorded for 1-, 2-, 3-, and 4-year-old cladodes, respectively. K came in third place with  $6.48 \pm 0.78$ ,  $8.10 \pm 1.12$ ,  $8.86 \pm 0.71$ , and  $4.57 \pm 0.40$  g/kg DM recorded for 1-, 2-, 3-, and 4-year-old cladodes, respectively. Na was not detected; it was either completely absent or present in minimal amounts. The maximum K, Ca, and Ba concentrations were found in cladodes of 2 and 3 years old. The mineral contents found in this work are different from those found by Astello-García et al. [11] for *Opuntia megacantha* variety Amarillo Platano, where K is the most abundant mineral (17.30–28.70 g/kg) followed by Ca (6.10–8.20 g/kg) and Na (2.60 g/kg). The median lethal dose (LD50) of Ba is 19.2 mg Ba<sup>2+</sup>/kg for a rat by intravenous administration [37]. Therefore, *Opuntia stricta* cladodes consumption should be done in moderation.

**3.9. Total Polyphenol Content.** The highest total polyphenol contents with no significant difference ( $P > 0.05$ ) were recorded for 2- and 4-year-old cladodes ( $164.50 \pm 2.03$  and  $164.97 \pm 3.22$  mg GAE/100 g DM, respectively). Their contents were significantly higher ( $P < 0.05$ ) than those of 1- and 3-year-old cladodes ( $157.98 \pm 5.66$  and  $133.86 \pm 2.90$  mg GAE/100 g DM, respectively) (Figure 3).

These polyphenol contents are higher than that reported by Elshehy et al. [38] for *Opuntia ficus-indica* cladodes (119.66 mg/100 g DM). However, they are significantly lower than those found by Ayadi et al. [39] reaching up 900 mg/100 g DM and those reported by Hadj Sadok [34] for *Opuntia ficus-indica* cladodes (ranging from 23.4 to 41.6 mg/100 g FM). By comparing to other fruits, the polyphenol contents of *Opuntia stricta* cladodes of different ages are lower than those recorded for ten Moroccan cultivars of pomegranate (varying between 63.28 and 116.83 mg GAE/100 g FM, corresponding to 4562.65 and 8725.09 mg GAE/100 g DM, respectively) [35, 36]. The antioxidant properties of polyphenols are well known for protecting against

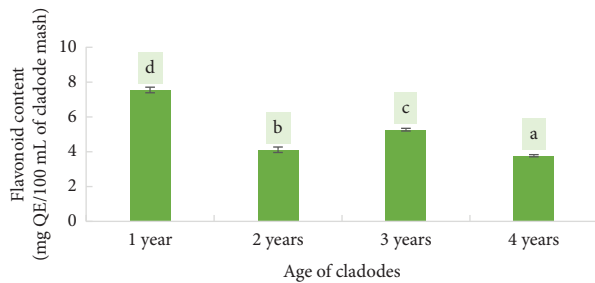


FIGURE 5: Flavonoid content of *Opuntia stricta* cladodes at four ages (QE: quercetin equivalent; means with the same letter do not differ significantly according to Scheffé test at  $P < 0.05$ ; error bars represent the standard deviation of means).

oxidative stress. Their abundance in *Opuntia stricta* cladodes could be exploited to develop a wide range of products, especially foods and cosmetics.

**3.10. Ascorbic Acid Content.** With the exception of the age gap between 2 and 3 years ( $P > 0.05$ ), the ascorbic acid contents obtained across cladodes of different ages revealed significant differences ( $P < 0.05$ ) (Figure 4).

The content of ascorbic acid was highest for 1-year-old cladodes ( $18.53 \pm 0.61$  mg GAE/100 g DM) and lowest for 4-year-old cladodes ( $8.09 \pm 1.34$  mg GAE/100 g DM). Overall, the vitamin C content of cladodes decreased significantly with age, which is in accordance with the change in pH and TTA of cladodes with age. The ascorbic acid contents recorded in this study are in concordance with those reported by Stintzing and Carle [22] for *Opuntia* spp. cladodes (ranging from 7 to 22 mg/100 g DM) but they are lower than that found by Hadj Sadok [34] for *Opuntia ficus-indica* cladodes (15 mg/100 g FM). According to Izuegbuna et al. [40], the vitamin C content in *Opuntia stricta* cladodes powder is very low (2.9 mg/100 g DM). Due to its high ascorbic acid content, *Opuntia stricta* cladodes might be a good source of natural antioxidants.

**3.11. Flavonoid Content.** Flavonoids are water-soluble phenolic antioxidants that are highly abundant in plant matrices [41]. Analysis of variance of flavonoid contents revealed significant differences between cladodes at four ages ( $P < 0.05$ ). The recorded contents vary from  $3.77 \pm 0.06$  to  $7.55 \pm 0.16$  mg QE/100 mL of cladodes mash. The youngest cladodes of 1 year old were found to have the highest flavonoid content, which significantly decreased with age (Figure 5). The contents found in this investigation are higher than those reported by Boutakiout [10], which vary between  $1.18 \pm 0.01$  and  $1.36 \pm 0.04$  mg rutin equivalent/100 mL in the cladode juices of *Opuntia ficus-indica* and *Opuntia megacantha*. According to this study, the flavonoid content of erect prickly pear fruit pulp is very low ( $0.98 \pm 0.3$  mg/100 g) [42]. Although, the total flavonoid content recorded by Bouzoubaâ et al. [43] for *Opuntia megacantha* fruit is in the range found in this study for the cladodes (5.02 mg QE/100 g FM).

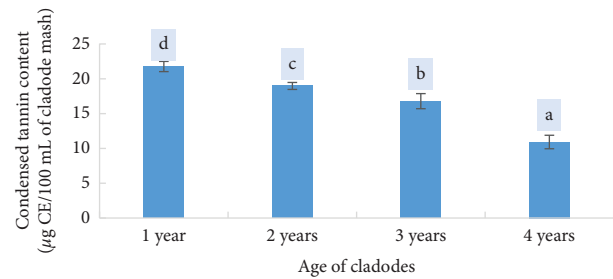


FIGURE 6: Condensed tannin content of *Opuntia stricta* cladodes at four ages (CE: catechin equivalent; means with the same letter do not differ significantly by Scheffé test at  $P < 0.05$ ; error bars represent the standard deviation of means).

**3.12. Condensed Tannin Content.** Condensed tannins are powerful antioxidants. They are phenolic compounds that precipitate proteins and cause astringency (a drying sensation in the mouth). According to the analysis of variance, tannins are minor compounds in *Opuntia stricta* cladodes, with significant differences between the cladodes at four different ages. The content of condensed tannins is inversely proportional to the age of the cladodes. The recorded values vary between  $10.92 \pm 0.97$  and  $21.76 \pm 0.73$  µg CE/100 mL of the cladodes mash for 4- and 1-year-old cladodes, respectively (Figure 6). The contents found in this study are lower than those found by Boutakiout [10] for *Opuntia ficus-indica* and *Opuntia megacantha* cladodes juices, ranging from  $12.10 \pm 0.21$  to  $18.23 \pm 0.36$  mg Trolox equivalent/100 mL.

**3.13. Betalains Content.** Betalains are coloured pigments, including betacyanins (red-violet pigments) and betaxanthins (yellow-orange pigments) [44]. Total betalains vary significantly ( $P < 0.05$ ) from  $4.93 \pm 0.60$  to  $12.48 \pm 0.49$  mg/100 g DM for 3- and 2-year-old cladodes, respectively. Betaxanthins were higher than betacyanins for cladodes at four ages (Figure 7). These contents of betalains are higher than those found by Du Toit et al. [45] for *Opuntia robusta* cladodes ( $2.92 \pm 1.32$  mg/100 g FM). Betalains are characteristic molecules of prickly pear fruits, according to Zenteno-Ramírez et al. [44]. Their contents in *Opuntia* spp. fruits vary from 4.4 mg/100 g DM for *Opuntia albicarpa* variety Blanca white fruits to 407.4 mg/100 g DM for *Opuntia robusta* red fruits. Fruit pulp of *Opuntia stricta* var. *dillenii* showed greater content of total betalains (444.77 mg/100 g FM) according to Gómez-López et al. [24].

**3.14. Photosynthetic Pigment Content.** The content of chlorophyll, known for its preventive role against cancer [46], varies significantly between cladodes at four ages ( $P < 0.05$ ). The recorded contents are in decreasing order:  $23.76 \pm 0.25$ ,  $14.03 \pm 0.02$ ,  $11.46 \pm 0.07$ , and  $5.55 \pm 0.17$  mg/100 g DM for ages 1, 3, 2, and 4 years, respectively (Figure 8).

The recorded carotenoid contents are  $5.98 \pm 0.08$ ,  $3.61 \pm 0.02$ ,  $2.53 \pm 0.01$ , and  $1.85 \pm 0.09$  mg/100 g DM for ages 1, 2, 3, and 4 years, respectively. These low contents differed significantly ( $P < 0.05$ ) between cladodes at the four ages



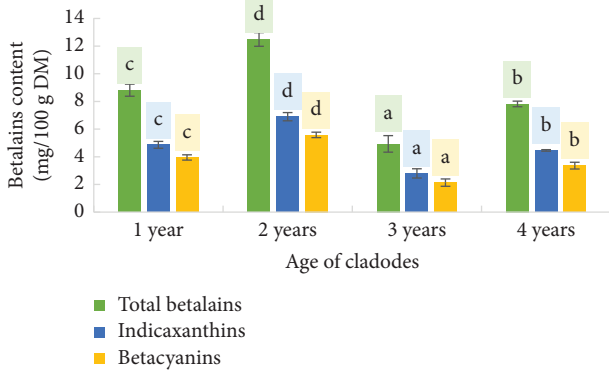


FIGURE 7: Betalains content of *Opuntia stricta* cladodes at four ages (DM: dry matter; means with the same letter do not differ significantly according to Scheffé test at  $P < 0.05$ ; error bars represent the standard deviation of means).

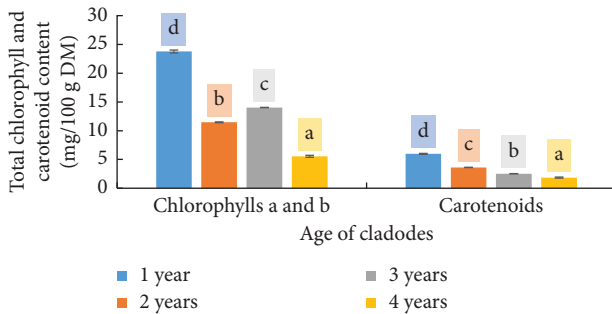


FIGURE 8: Total chlorophyll (a and b) and carotenoid contents of *Opuntia stricta* cladodes at four ages (DM: dry matter; means with the same letter do not differ significantly according to Scheffé test at  $P < 0.05$ ; error bars represent the standard deviation of means).

(Figure 8). Then, chlorophyll and carotenoid contents decrease significantly as cladodes get older. Taking into account the organoleptic and nutritional qualities and health benefits, young cladodes of *Opuntia stricta* would be recommended. The chlorophyll contents recorded in this work are lower than those found by Guevara et al. [12] where total chlorophyll content in cladodes was estimated at 12.5 mg/100 g FM, taking into consideration that the content of chlorophyll (a) is 9.5 mg/100 g exceeding that of chlorophyll (b) estimated at 3.0 mg/100 g. Hadj Sadok [34] stated that the chlorophyll contents in cladodes of *Opuntia ficus-indica* form *inermis* at different stages of growth vary slightly from 3.45 to 4.32 mg/100 g FM. The carotenoid contents found in this study for *Opuntia stricta* cladodes are higher compared to those recorded by Hadj Sadok [34] for *Opuntia ficus-indica*, which vary between  $0.077 \pm 0.06$  and  $0.042 \pm 0.04$  mg/100 g FM. These results also show that the carotenoid contents of cladodes remain slightly close to those of other vegetables, where the levels vary from 0.01 to 9 mg/100 g FM [47].

**3.15. Antioxidant Activity.** The highest-free-radical scavenging activity of *Opuntia stricta* cladodes extract determined by the DPPH test was recorded for 4-year-old

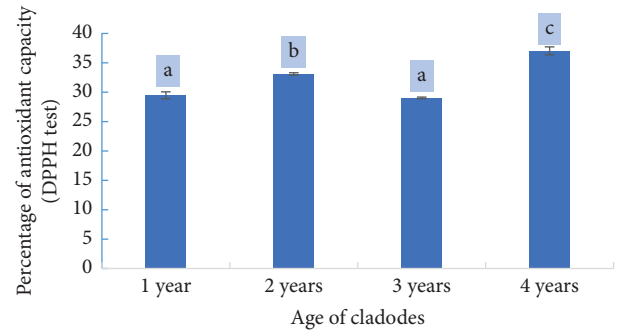


FIGURE 9: Antioxidant activity of *Opuntia stricta* cladode extracts at four ages (means with the same letter do not differ significantly according to Scheffé test at  $P < 0.05$ ; error bars represent the standard deviation of means).

TABLE 4: Inhibitory concentration 50 (IC50) of *Opuntia stricta* cladodes at four ages.

Age (years)	IC50 (g/mL)
1	0.66
2	0.63
3	0.74
4	0.56

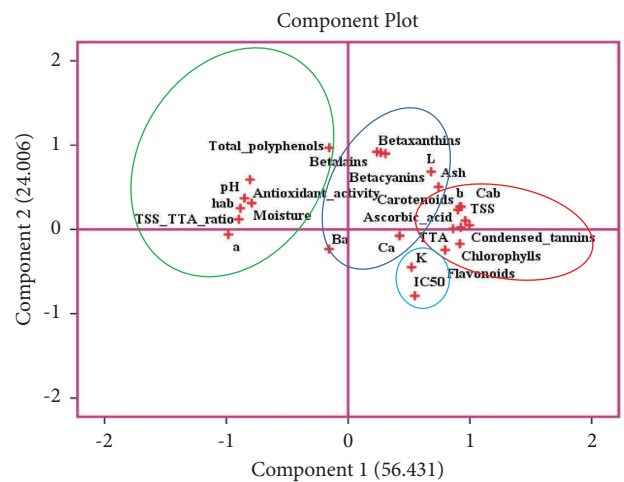


FIGURE 10: Principal component analysis: factor loading plot of physical, physicochemical, and biochemical parameters of *Opuntia stricta* cladodes at four ages along components 1 and 2 (TSS: total soluble solids; TTA: total titratable acidity).

cladodes, followed by 2-year-old cladodes, with percentages of inhibition of  $37.04 \pm 0.68\%$  and  $33.12 \pm 0.20\%$ , respectively ( $P < 0.05$ ). The lowest proportion of antioxidant capacity was recorded for 1- and 3-year-old cladodes (Figure 9). These percentages of inhibition are close to the value of 40.38% found by Elshehy et al. [38], and they are higher than the value of 12.7% reported by Astello-Garcia et al. [11]. Du Toit et al. [44] recorded inhibition percentages ranging from 83.77% to 95.53% for *Opuntia robusta* cladodes.

The 4-year-old cladodes have the lowest inhibitory concentration 50 (IC50), with 0.56 g/mL of cladodes extract able of scavenging 50% of DPPH• radicals. The antioxidant

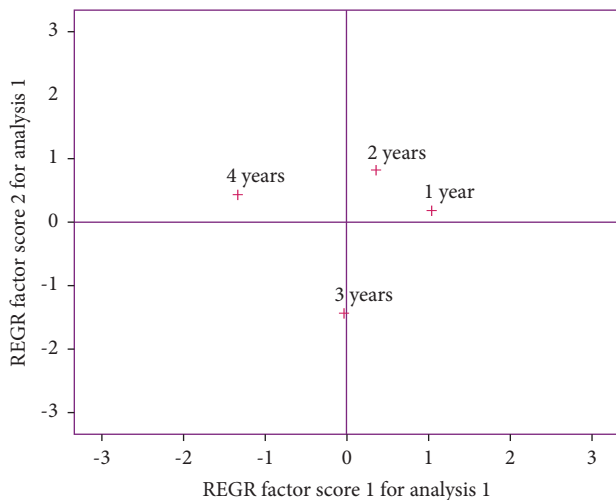


FIGURE 11: Principal component analysis score plots of cladode age distribution of *Opuntia stricta* based on physical, physicochemical, and biochemical parameters.

capacity of *Opuntia stricta* cladodes mash can be described as strong. Moreover, these values clearly show that 4-year-old cladodes show more potent antiradical activity than cladodes of other ages (Table 4). The IC50 values found in this work are higher than those recorded by Yeddes et al. [48] for two Tunisian *Opuntia ficus-indica* forms (spiny and thornless) and *Opuntia stricta* fruit methanol extract for both peel and pulp (ranging from  $0.40 \pm 0.03$  to  $0.57 \pm 0.02$  mg/mL).

**3.16. Principal Component Analysis (PCA).** PCA, performed on the studied physical, physicochemical, and biochemical parameters related to *Opuntia stricta* cladodes, is presented in Figures 10 and 11. In Figure 10, the first two PCA components (C1 and C2) explain 56.43% and 24.1%, respectively, accounting for 80.44% of the overall variation. According to the rotation factor loading plot (Figure 10) and the distribution of the cladode ages in the score plots (Figure 11), the studied parameters can be categorised into four groups. The parameters: chlorophylls, carotenoids, TSS, condensed tannins, vitamin C, chromaticity  $C_{ab}^*$ , and colour parameters  $L^*$  and  $b^*$  are higher for 1-year-old cladodes in comparison to other ages. The parameters ash, Ca, Ba, TTA, and betalain with its two types, indicaxanthin and betacyanin, presented high values for 2-year-old cladodes. K content and IC50 were the highest for 3-year-old cladodes. The parameters: antioxidant activity, total polyphenols, pH, moisture, TSS/TTA ratio, chrominance  $h_{ab}$  and colour parameter  $a^*$ , are present with high values for 4-year-old cladodes. The parameters: chlorophylls, carotenoids, TSS, condensed tannins, vitamin C, IC50, chromaticity  $C_{ab}^*$  and colour parameter  $b^*$ , are negatively correlated with the parameters: antioxidant activity, total polyphenols, pH, moisture, TSS/TTA ratio, and chrominance  $h_{ab}$ . Antioxidant activity is strongly correlated with the content of total polyphenols and betalains. IC50 is negatively correlated with antioxidant activity. According to Figure 11, where factors 1 and 2 summarise the segregation of ages according to the

physical, physicochemical, and biochemical parameters of *Opuntia stricta* cladodes, the great distinction of 3-year-old cladodes from other ages for some of the studied parameters would be due to the great physiological changes of cladodes of this age related to their great buds emission.

## 4. Conclusions

The results of the physicochemical analyses performed on *Opuntia stricta* cladodes of four different ages clearly demonstrate the richness of 1- and 2-year-old cladodes in total soluble solids, total organic acids, and ash content compared to 3- and 4-year-old cladodes. Cladodes aged 4 years stood out from the rest in terms of flavour, having the highest TSS/TTA ratio. The analysis of minerals revealed calcium richness. Hence, approximately 170 g of fresh cladodes may provide the daily nutritional requirements for calcium. Concerning bioactive compounds, *Opuntia stricta* cladodes showed high betalain levels, close to some prickly pear fruits. 2-year-old cladodes are the richest in betalains, with a dominance of indicaxanthins (yellow pigments). The content of phenolic compounds remains acceptable. Condensed tannins are not very abundant in *Opuntia stricta* cladodes. These cladodes also contain very high amounts of vitamin C, especially 1-year-old cladodes. Based on all the studied physicochemical and biochemical characteristics of *Opuntia stricta* cladodes, it can be concluded that these cladodes can be a good source of nutrients and natural antioxidants. Therefore, *Opuntia stricta* has a great potential to be valorised in drylands through the production of food and cosmetics using cladodes. Furthermore, the plantation of this species as a crop or in dryland restoration projects will contribute in improving the environment and livelihood of the local population.

## Data Availability

All data generated or analysed during this study are included within this article.

## Conflicts of Interest

The authors declare that they have no conflicts of interest regarding the publication of this article.

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