ISSN 2347-4289

Effect Of Season On Proximate Composition Of Cladode Juice Of Two Species Of Cactaceae

A. Boutakiout, D. Elothmani, M. Mahrouz, H. Hanine

UPSP GRAPPE, Groupe ESA, SFR QUASAV 4207, 55, rue Rabelais BP 30748, F-49007 Angers Cedex 01, France.
Laboratory of Biointerface and Bioprocedes, Faculty of Sciences and Techniques, university of Sultan Moulay Slimane, Beni Mellal, Morocco.
Laboratory of Research and Innovation, Sutainable Developpement and Green Chemistry Expertise, University of Cadi Ayyad, Faculty of Sciences Semlalia, Marrakech, Morocco.

Abstract: This study evaluates the effects of harvesting seasons on proximate composition, polyphenols, and antioxidant activity of cladode juice of the two species Opuntia ficus-indica (spineless) and Opuntia megacantha (spiny) selected in Morocco. Proximate composition showed that cladode juice is rich in ascorbic acid (22.88 ± 0.62 mg /100 mL) and potassium (409.35 mg /100 mL) but poor in sugar (1.45 ± 0.03 g/100 mL). Total phenolics and antiradical activity gives high values ranging from 455.65 ± 7.63 to 542.70 ± 1.35 µg GAE/mL and from 1.78 ± 0.03 to 4.10 ± 0.02 µmol TE/mL respectively. The highest analyses values were recorded in summer. Correlation analyses indicated that there were two significant relationships ($p<0.05$) between analyses and the month of harvest and a second between analyses and cultivars. Results of the present study confirmed that components of cladode juice whose phyto-chemistry and phyto-pharmacology should be investigated further in order to detect possible phyto-therapeutic uses.

Keywords: cladode juice, proximate composition, minerals, polyphenols, antioxidant activity.

1 Introduction

Natural products and health foods have recently received a lot of attention both by health professionals and the common population for improving overall well-being, as well as in the prevention of diseases. They are notable by the fact that they are locally available but universally erratic and much related information is also limited but they have a health-promoting benefit [28]. The Opuntia genus also known as prickly pear cactus (Opuntia ficus indica; Opuntia spp.,Cactaceae) contains around 300 species. It belongs to the Cactaceae or cactus family which contains approximately 130 genera and 1500 species [25]. They are widely distributed on arid and semi-arid regions. The origin of this crop comes from the United States, Mexico, and South America. Its cultivation was spread on other continents [9]. The two species Opuntia ficus-indica and Opuntia megacantha (Fig.1) are different on their young edible stems called cladodes replacing leaves in their photosynthetic function and which are spineless and spiny respectively [36]. In Morocco, cladodes are generally used as animal feed. We can also have other products like jam or pickles and candied nopales. Prickly pear has also been planted on steep slopes to control erosion [37].

(a) **(b)**

Fig. 1 *Cladodes of prickly pear: (a) spineless species; (b) spiny species.*

The cactus cladodes are mainly constituted by a heteropolysaccharide with a molecular weight ranging from 104 to 106 g/mol [5]. In addition, the cactus contains chemicals exhibiting potent antioxidant activity and further functional properties [23]; [36]. Theses bioactive compounds have a considerable health benefit [15]. Extracted compounds from cladodes shows a number of pharmalogical actions including antioxidant capacity, antiviral properties, antispermatogenic properties, analgesic, antiulcerogenic, hypoglycemic, antidiabetic, antihyperlipidemic, cholesterol-lowering, and anti-atherogenic effect [36]. They were also valued for their anti-inflammatory activity in treating edema, arthritis, whooping cough, and for preventing wound infection [10]. There are increasing concerns and recommendations for consumers to use natural antioxidants from plant sources since the use of synthetic antioxidants in food (butylated hydroxytoluene BHT, butylated hydroxyanisole BHA) have been restricted because some of them have been found to be toxic and carcinogenic. The frequent consumption of fruits and vegetables rich in natural antioxidants was reported in many epidemiological studies to lower the incidence of certain types of cardiovascular diseases, diabetes and cancer [14]. Moreover, it has been well documented that natural polyphenolic compounds have a close relationship within those properties [27]. Prickly pear contains a wide variety of phenolic compounds which are found to be well correlated with antioxidant potential [36]. Those natural antioxidants can be phenolic compounds (tocopherols, flavonoids, and phenolic acids), nitrogen compounds (alkaloids, chlorophyll derivatives, amino acids, and amines), minerals (selenium and zinc), carotenoids, and vitamins [38]. The nutritional and pharmacological benefits of the different parts of the prickly pear, in addition to its increasing importance at the industrial level, have motivated our investigation about the chemical contents of the cladode juice of the two species Opuntia ficus-indica and Opuntia megacantha, this study was carried out to evaluate the proximate composition, antioxidant activity, and total polyphenolic content in order to explore the nutritional properties of cladode juice because there is no study yet on cladode juice of the two species Opuntia ficus-indica and Opuntia megacantha.

2 Materials and Methods

2.1 Plant Materials

The materials used for this study are shown in Fig.1. Two different cladodes (spineless and spiny) were collected from the rural town of Ouled Dlim in Marrakech (South of Morocco). The average temperature of this area is between 20 and 22°C. The warmest month is reaching 48°C in August and the coldest 3°C in January. The average rainfall is 199.7 mm/year. Cladodes of the two species Opuntia ficus-indica (spiny) and Opuntia megacantha (spineless) were collected in three periods during the year, in intermediate, hot, and cold season corresponding to the following months: march (2013), august (2013), and january (2014). Cladode collection was performed in the morning. Cladodes less than a year were selected at random. They were cut to 1 meter height from the ground. The dimensions were chosen according to the following conditions: length between 15 and 25 cm and width between 9 and 12 cm. It is a medium which corresponds to “napolitos” (3-8 months). The quantity harvested was 20 kg for each period. Batches of cladodes were stored at -20°C before analyses.

2.2 Chemicals and Reagents

Folin–Ciocalteu’s phenol reagent, sodium hydroxide, sodium carbonate, potassium and sodium tartrate, copper II sulfate pentahydrate, tannic Acid, potassium iodate, aluminum chloride, rutin, sulfuric acid, phenol, glucose, iodine, Trolox and methanol were purchased from R&M Chemicals (Essex. UK). The DPPH (2,2-diphenil-1-picridazil) was purchased from the Fluka company (Switzerland).

2.3 Extract Preparation

Extraction of juice was made by grinding and centrifuging cladodes. The spines were removed and cladodes were cut into cubes of 2 cm. They were crushed at 10000 rpm for 20s, and then centrifuged at 15000 rpm at 4°C for 20 min. The supernatant was recovered and the clarified juice obtained was stored at -20°C prior to analyses. The juice yield was calculated relative to the initial mass of cladodes.

2.4 Proximate analysis

Moisture was determined from sample weight loss after oven drying the juice at 110°C for 4 h. Ash was calculated after heating the sample at 550 °C for 3 h. Titrable acidity was measured by titration of 25 mL of juice in the presence of sodium hydroxide (0.25 N) to a pH of 8.1. The pH was measured using a pH meter (S20 SevenEasy, Mettler Toledo). The degrees Brix was measured using an electronic refractometer (KYOTO, RA-130).

2.4.1 Proteins

The total protein content was performed by the method of Lowry et al.(1951) with some modifications [24]. The juice was diluted with distilled water to 1/20. An alkaline pretreatment solution was prepared by weighing 20g of sodium hydroxide, 100g of sodium carbonate, 2g of potassium and sodium tartrate, and 0.5g of copper II sulfate pentahydrate then completing by distilled water to 1000 mL. The working solution was made by mixing 1 mL of the diluted juice with 1 mL of the pretreatment solution and it was allowed to stand for 10 min at room temperature. Then 4 mL of Folin reagent diluted to 1/8 was added. The solution was incubated for 5 min at 55 ± 1°C and cooled in cold water for 30 min. The measurement of the absorbance was at 670 nm using a spectrophotometer (Power wave XS BIOTECK). The change in absorbance between the final reading selected and the blank reading was calculated for each sample. The concentration of Bovine Serum Albumin (BSA) used as a standard was between 1 and 20 mg/100mL. Results were expressed in mg BSA Equivalent/mL. The experiment was repeated three times.

2.4.2 Condensed Tannins

This assay was performed according to the colorimetric method of Folin Denis by Joslyn (1970) with some modifications [17]. The juice was diluted with a water-methanol mixture (2:3) to 1/10. The working solution was made by mixing 0.5 mL of the diluted juice with 2.5 mL of Folin and 5 mL of sodium carbonate (7.5%). The mixture was allowed to stand for 30 min at room temperature in the dark. Then the solution was incubated for 5 min at 55 ± 1°C and cooled in cold water for 30 min. The measurement of the absorbance was at 760 nm using a spectrophotometer (Power wave XS BIOTECK). The change in absorbance between the final reading selected and the blank reading was calculated for each sample. The concentration of tannic acid used as standard was between 10 and 100 mg/L. Results were expressed in mg Tannic Acid Equivalent/mL. The experiment was repeated three times.

2.4.3 Water Soluble Tannins

Water soluble tannins are determined by the method of Willis and Allen (1998) with some modifications [39]. The juice was diluted with a water-methanol mixture (2:3) to 1/10. The working solution was made by mixing 0.5 mL of the diluted juice with 2.5 mL of potassium iodate solution (2.5%) and it was allowed to stand for 4 min at room temperature in the dark. The measurement of the absorbance was at 550 nm using a spectrophotometer (Power wave XS BIOTECK). The change in absorbance between the final reading selected and the blank reading was calculated for each sample. The concentration of tannic acid used as standard was between 10 and 100mg/L. Results were expressed in mg Tannic Acid Equivalent/mL. The experiment was repeated three times.

2.4.4 Flavonoids

Total flavonoids content was determined using the method of Lamaison and Carnat (1990) with some modifications [21]. The juice was diluted with a water-methanol mixture (2:3) to 1/100. The working solution was made by mixing 1 mL of diluted juice with 1 mL of aluminum chloride (2%). The measurement of the absorbance was at 430 nm using a spectrophotometer (Power wave XS BIOTECK). The change in absorbance between the final reading selected and the blank reading was calculated for each sample. The concentration of rutin used as the standard was between 10 and 100 mg/L. Results are expressed in mg Equivalent Rutin/mL. The experiment was repeated three times.

2.4.5 Sugars

The determination of sugars was realized by the method of Dubois (1965) with some modifications [12]. The working solution was made by mixing 1 mL of juice with 1 ml of

phenol (10 %) and 5 mL of concentrated sulfuric acid. The measurement of the absorbance was immediate at 430 nm using a spectrophotometer (Power wave XS BIOTECK). The change in absorbance between the final reading selected and the blank reading was calculated for each sample. The concentration of glucose used as standard was between 10 and 100mg/L. Results are expressed in mg Equivalent Glucose/mL. The experiment was repeated three times.

2.4.6 Pectin

The determination of pectin was made by mixing 2 mL of juice with 6 mL of ethanol (80%) in an acidic medium (pH = 1.5). Then the mixture was centrifuged at 8000 rpm for 15 min, and then the pellet was recovered in an oven set at 50 °C. The percentage of pectin in the juice was calculated by mass difference.

2.4.7 Ascorbic Acid

The determination of ascorbic acid was realized according to the iodometric method. The working solution was made by mixing 5 mL of the juice with 5 mL of iodine (0.005 M) and some drops of starch paste. Titration was carried out with the addition of thiosulfate (0.005 M) until disappearance of the dark color.

2.4.8 Minerals

Minerals quantified in this analysis are the macro-elements: Ca, K, Mg, Na. Analysis was performed by ICP Atomic Emission Spectrometry (AES Ultima 2-Jobin Yyon).

2.5 Determination of the Free DPPH Radical Scavenging Effect

The DPPH assay was done according to the method of Brand-Williams et al. (1994) prepared with some modifications [7]. Samples were analyzed in methanol extract by mixing 2g of juice with 22 mL of methanol and homogenized. The stock solution of DPPH• was prepared in methanol with a concentration of 60 µM. The solution was stirred for 30 min protected from light. The working solution was obtained by mixing 0.1 mL of cladode extract with 3.9 mL of the DPPH solution. The samples were allowed to react for 8h in the dark and the decrease in absorbance at 515 nm was measured using a spectrophotometer (Power wave XS BIOTECK). The blank contains 0.1 mL of methanol instead of extracts. The experiment was carried out in triplicate. Results are expressed in µmol Trolox Equivalent (TE)/mL. The concentration of Trolox as standard was between 10 and 500 µM in methanol. DPPH• radical scavenging capacity was calculated using following equation.

$$\mathbf{DPPH^{\bullet}\ scavenging\ effects}\ \% = \frac{\mathbf{A_{(blank)}} - \mathbf{A_{(sample)}}}{\mathbf{A_{(blank)}}} \times \mathbf{100}$$

Where Ablank is the absorbance of the blank and Asample is the absorbance of the sample.

2.6 Total Phenolics Content

Total phenolics were determined by Folin-Ciocalteu method of Singleton et al. (1999) with some modifications [34]. Samples were analyzed in methanol extract by mixing 2g of juice with 22 mL of methanol and homogenized. The stock solution included 1:10 of Folin-Ciocalteu reagent, 60g/L of sodium carbonate. The homogenates were then centrifuged at 15 000 rpm for 20 min. The supernatants were recovered and stored at -20°C until analyses. The working solution was prepared by mixing 1.5 mL of diluted Folin-Ciocalteu reagent with 0.2 mL of cladode extract. Then let the mixture stand for 5 min and add 1.5 mL of sodium carbonate. The absorbance was measured at 725 nm after 30 min of reaction. The change in absorbance between the final reading selected and the blank reading was calculated for each sample. Results are expressed in µg Gallic acid Equivalent/mL. The concentration of Gallic acid as standard was between 10 and 500 µM in methanol. The experiment was carried out in triplicate.

2.7 Statistical Analysis

Statistical analyses were conducted to compare the results obtained in the replications and the data were expressed as the mean ± standard deviation (SD). All the analysis was performed by Statistical Analysis System (SAS) software 917 SAS Institute Cay N.C. (USA). Using analysis of variance (ANOVA) and differences among means were determined for significance at $p < 0.05$ using the PROC GLM procedure.

3 Results and Discussion

3.1 Yield and Proximate Composition of the Juice

The juice yield of the two species Opuntia ficus-indica and Opuntia megacantha obtained from their cladodes showed that both varieties are rich in water (Table 1).

Table 1 Juice yield of the two species Opuntia ficus-indica (spineless) and Opuntia megacantha (spiny).

Months	Juice yield (%)	
	Spineless	Spiny
March	51.96±0.00[a]	63.39±0.00[a]
August	20.83±0.00[c]	35.58±0.00[c]
January	38.25±0.00[b]	45.19±0.00[b]

[a,b,c,d] *Values having same letter within the column did not differ significantly from each other according to LSD test at $p < 0.05$.*

The yield values varied largely among the two species, where cladodes obtained in march achieved the highest yield and precisely with the spiny form (63.39 %), cladodes obtained in august had the lowest yield and it was observed with the spineless form (20.83%). We note that the spiny form registered the highest values of juice yield during the three seasons. In a study of citrus, mandarin juice yield ranged from 42.85 % to 60.74 %, orange from 43.53 % to 50.79 %, lemon 40.39 %, and grapefruit 40.04% [40]. Indeed, we can compare cladode juice yield with citrus juices yield except in summer where we observe a very low yield of cladode juice (20.83% –35.58%). We have to know that rain is the only source of water for these plants so it influences their ability to store water. In those cultivars, 3 months of drought decreases photosynthesis night phase (down of 73% of nocturnal acid accumulation in the chlorenchyma) and abolishes sweating, but also 27% of the water in the chlorenchyma and 61% of water in

parenchyma are lost during this period [16]. It was reported that spine functions include mechanical protection from herbivores, reflection of light, reducing the surface area of the cortex in the sun exposure, raising water retention and condensing fog [22]; [36]. In fact, the juice yield was higher for the spiny cladodes than the spineless. Proximate compositions of cladode juice are shown in Table 2, 3 and 4. Table 2 summarized general composition of spiny and spineless cladodes species.

Table 2 Relevant chemical characteristics of cladode juice of the two species Opuntia ficus-indica and Opuntia megacantha

Months	Species	Water (g/100g of juice)	Ash (g/100g of juice)	Degrees Brix (°Bx)	pH	Titratable acidityEquivalent citric acid (g/L)
March	Spineless	95.68 ± 0.07^{a}	0.82 ± 0.03^{c}	5.5±0.0^{c}	4.68±0.01^{a}	1.45±0.01^{c}
	Spiny	95.62 ± 0.01^{a}	0.75 ± 0.01^{d}	5.1±0.0^{c}	4.74±0.01^{a}	1.03±0.01^{d}
August	Spineless	91.00 ± 0.26^{c}	1.74 ± 0.04^{a}	11.0±0.0^{a}	4.77±0.01^{a}	1.88±0.01^{b}
	Spiny	91.26 ± 0.04^{c}	1.67 ± 0.00^{a}	10.7±0.0^{a}	4.74±0.01^{a}	2.35±0.01^{a}
January	Spineless	95.13 ± 0.11^{a}	0.79 ± 0.02^{d}	6.1±0.0^{b}	4.57±0.01^{a}	1.20±0.01^{d}
	Spiny	94.24 ± 0.11^{b}	0.96 ± 0.02^{b}	7.2±0.0^{b}	4.49±0.01^{a}	1.52±0.01^{c}

^{a,b,c,d} *Values having same letter within the column did not differ significantly from each other according to LSD test at p<0.05.*

High moisture, ash, and TSS (Brix) contents were observed for both varieties of cladodes, but more important for the spineless species. However, the two varieties of cladodes exhibit a higher acidity value, and low pH value. This low acidity value and low pH value could be explained by the presence of many organic acids as malic, citric and oxalic acids as salts form [36]. These results were in concordance with previously works of [32], a pH value of 4.6 was registered for cladodes of O. ficus indica f. amylocea and O. ficus indica f. inermis cultivated in Mexico. Indeed, the composition of cladodes varieties depending on the edaphic factors at the cultivation site, the season and the age of the plant [30]. Therefore, the respective values vary both among species and varieties and should not be taken as absolute values [32]. Water content ranged from 95.68 ± 0.07 to 91.00 ± 0.26 g/100g. Spiny cladodes have recorded the lowest values of water content during the three seasons and precisely in august (91.00 ± 0.26 g/100g). In general, for cactus stems of Opuntia the water content is between 88 – 95% [36]. Ash ranged from 1.74 ± 0.04 to 0.75 ± 0.01 g/100g. The highest values of ash were observed for the spiny form and more in august. We can observe that those two responses (water content and ash) are inversely proportional which can explain that spiny cladodes are rich in bimolecular compounds. We had the same results for the °Brix, pH and titrable acidity, spiny cladodes and cladodes collected in august had the highest values. The pH was between 4.49 – 4.77, therefore the juice has a medium acidity. In addition, these results demonstrate the good nutritional potential of this Cactaceae species and establish the basis for further studies to test the possibilities of the use the species as an alternative feed resource for human and animals.

Table 3 Proximate composition of cladode juice of the two species Opuntia ficus-indica and Opuntia megacantha

Month	Species	Proteins (g /100mL)	Sugars (g/100mL)	Pectins (%)	Condensed tannins (mg/100mL)	Hydosoluble tannins (mg/100mL)	Flavonoids (mg/100mL)	Ascorbic acid (mg/100 mL)
March	Spineless	0.18 ± 0.02^{d}	0.76 ± 0.01^{b}	0,10^{f}	12,10 ± 0,21^{f}	1,28 ± 0,04^{a}	1,24 ± 0,01^{b}	22,88 ± 0,62^{a}
	Spiny	0.27 ± 0.01^{c}	0.66 ± 0.01^{b}	0,30^{e}	13,34 ± 0,36^{e}	1,36 ± 0,15^{a}	1,22 ± 0,00^{b,c}	19,36 ± 3,11^{b}
August	Spineless	0.50 ± 0.04^{a}	1.45 ± 0.03^{a}	2,25^{a}	16,90 ± 0,43^{b}	1,32 ± 0,05^{a}	1,36 ± 0,04^{a}	17,60 ± 0,62^{c}
	Spiny	0.48 ± 0.02^{a}	1.30 ± 0.03^{a}	1,85^{b}	18,23 ± 0,36^{a}	1,33 ± 0,15^{a}	1,35 ± 0,05^{a}	19,36 ±1,87^{b}
January	Spineless	0.34 ± 0.03^{b}	0.85 ± 0.02^{b}	0,45^{d}	14,96 ± 0,13^{c}	1,24 ± 0,02^{a}	1,21 ± 0,02^{b,c}	17,60 ±1,87^{c}
	Spiny	0.40 ± 0.04^{b}	0.86 ± 0.02^{b}	0,95^{c}	14,07 ± 0,14^{d}	1,30 ± 0,13^{a}	1,18 ± 0,01^{c}	22,00 ± 0,65^{a}

^{a,b,c,d,e,f} *Values having same letter within the column did not differ significantly from each other according to LSD test at p < 0.05.*

Results showed that cladodes harvested in august has the highest values for most of analyses (Table 3). Crude protein content ranged from 0.18 ± 0.02 to 0.50 ± 0.04 g /100 mL. It was reported that on a fresh weight basis of spineless Opuntia cladode, protein content was between 0.1 and 1 g /100g and the major amino acid detected was glutamine, followed by leucine, lysine, valine, arginine, phenylalanine and isoleucine [36]. Total sugar content ranged from 1.45 ±

0.03 to 0.66 ± 0.01 g/100 mL. It was reported that for fresh cladode weight it reaches 0.32 g/100g [36]. Total sugar content of Moro and Sanguinello orange juice reached 10.3 and 10.9 g/100mL respectively [18], apple 9.95 g/100 mL, grape 16.1 g/100 mL, pineapple 11.6 g/100 mL, and tomato 3.86 g/ 100 mL [8]. We notice that cladode juice is poor in sugar compared to other juices. It makes cladodes a low-calorie food with 27 kcal/100g [36]. Pectin values content reached 2.25 %, theses pectin, partially responsible for the viscosity of the juice of cladode. We can compare it with apple juice where the values range from 2.62 to 2.94 % [2]. Condensed tannins are more present in cladode juice (18, 23 ± 0, 36 mg/100 mL) than hydrosoluble tannins (1.36 ± 0.15 mg/100 mL). The same thing was noticed in pomegranate juice for condensed and hyrosoluble tannins (43.20 ± 0.70 and 10.80 ± 0.30 respectively) [26]. Total flavonoids ranged from 1.36 ± 0.04 to 1.18 ± 0.01 mg/100 mL. In grape juice higher values were reported (7.24 mg/100mL) [11]. Ascorbic acid ranged from 17.60 ±1.87 to 22.88 ± 0.62 mg/100mL. It was reported that the average of ascorbic acid in Opuntia cladodes was between 7 and 22 mg/100 mL [36]. In other juices higher and lower values were reported: orange (38.30 mg/100 mL), apple (11 mg/100 mL), grape (Traces), pineapple (9.5 mg/100 mL), and tomato (16.6 mg/100 mL) [10]. We can conclude that juice cladode contains a great amount of ascorbic acid compared to other juices.

Table 4 Mineral content of cladode juice of the two species Opuntia ficus-indica and Opuntia megacantha

Month	Species	Ca (mg/100 mL)	K (mg/100 mL)	Mg (mg/100 mL)	Na (mg/100mL)
March	Spineless	21.43^{d}	51.88^{e}	4.52^{f}	0.02^{f}
	Spiny	258.78^{a}	156.02^{c}	91.31^{a}	0.46^{b}
August	Spineless	2.67^{f}	44.23^{f}	9.20^{e}	0.04^{e}
	Spiny	4.59^{e}	409.35^{a}	86.23^{b}	1.87^{a}
January	Spineless	29.71^{c}	155.57^{d}	44.80^{d}	0.17^{d}
	Spiny	45.37^{b}	162.88^{b}	51.33^{c}	0.20^{c}

^{a,b,c,d,e,f} *Values having same letter within the column did not differ significantly from each other according to LSD test at p < 0.05.*

Results showed that mineral content in juice cladode varies between seasons and species (Table 4). Potassium is the main mineral amounting to about 409.35 mg/100 mL of juice present in spiny cladodes harvested in august. This value is higher than the average amount of potassium in Opuntia cladodes (166 mg/ 100g) [36]. It was reported that the amount of potassium in apple juice was 106.80 mg/100mg (ABID et al., 2014), black currant juice 208.73 mg/100 mg, bilberry juice 92.96 mg/100 mg, and black raspberry juice 177.93 ± 8.21 mg/100g [19]. We observe that calcium and magnesium have approximately the same amount in juice cladode. But, the poor component was registered for sodium (0.025 mg / 100mL). The average content of calcium in Opuntia cladode is 93 mg/100mg while magnesium was not detected [36]. In apple juice it was registred 2.95 mg / 100g of calcium, 18.55 mg / 100g of magnesium, and 34.40 of sodium [1]. It was reported that in pulp, potassium is present at 161 mg/100 g, exceeding the concentration of other minerals like calcium and magnesium [13]. In fact, comparison between cladode juice and other juices showed that cladode juice is rich in potassium had a medium amount in Ca and Mg but poor in Na. These results demonstrate the good nutritional potential of this Cactaceae species and establish the basis for further studies to test the possibilities of the use the species as an alternative feed resource for human and animals.

3.2 Antioxidant Activity and Polyphenols Content

Antioxidant activity and polyphenols content was measured from a single extract three times to test the reproducibility of the assay (Table 5).

Table 5 Antioxidant activity and polyphenols content of juice cladodes of the two species: Opuntia ficus-indica and Opuntia megacantha.

Months	Species	DPPH (µmol TE/mL)	Polyphenols (µg GAE/mL)
March	Spineless	1.78±0.03^{d}	455.65±7.63^{b}
	Spiny	1.78±0.03^{d}	464.90±7.42^{b}
August	Spineless	3.58±0.03^{ab}	542.70±1.35^{a}
	Spiny	4.10±0.02^{a}	542.57±1.47^{a}
January	Spineless	2.70±0.04^{c}	523.90±1.85^{a}
	Spiny	3.14±0.06^{bc}	524.19±2.79^{a}

^{a,b,c} *Means within a column for each type of assay that have different letters are significantly different (p < 0.05).*

Polyphenols content ranged from 455.65±7.63 to 542.70±1.35 µg GAE/mL. The Highest value was registered in august (542.70±1.35 µg GAE/mL). Cladode Juice collected in january did not present a significantly difference (524.19±2.79 GAE/mL) but in march a significant variation was noticed (464.90±7.42 µg GA/mL). It was reported that total phenolics content for other juice fruits are: apple (296.3 ± 6.4 µg GAE/mL), banana (90.4 ± 3.2 µg GAE/mL), blackberry (26.7–452.7 µg GAE/mL), blueberry (261–587 µg GAE/mL), mango (6.25 ± 0.05 µg GAE/mL), pineapple (94.3 ± 1.5 µg GAE/mL), and strawberry (160 ± 1.2 µg GAE/mL) [4]. Indeed, cladode juice is rich in polyphenols compared to other fruit juices. Antioxidant activity of juice cladodes in methanol extract was slow and take more time compared to other juices or extracts. Determination of the end of the reaction is the most important step. The semi plate that appears over time informs us that the reaction is finished [7]. Determining IC50 which is widely used for the

DPPH assay to express the antioxidant activity could not be realized because the reaction is very slow and does not reached 50% of radical scavenging of DPPH•. Antioxidant activity of phenolic acids and their esters depends on the number of hydroxyl groups in the molecule that would be strengthened by steric hindrance [31]. The chemical properties of polyphenols in terms of the availability of the phenolic hydrogens as hydrogen donating radical scavengers predict their antioxidant activity [29]. Plant phenolic compounds react with proteins and enzymes under formation of covalent bonds [20]. These reactions diminish the antioxidant activity of phenolic compounds. Nevertheless, a covalent attachment to proteins means generally a loss of antioxidant activity of polyphenols like quercetin [33]. Such interactions between proteins and phenols can also take place in vivo. In this respect it was reposted that the plasma proteins can mask the antioxidant activity (TEAC assay) of phenolic compounds (quercetin, rutin, catechin, and 7-mono-hydroxyethylrutoside) [3]. Analyzes of antioxidant activity in cladode juice take time to react knowing they are rich in polyphenols. Extraction compounds and analyzing their antioxidant activities and their interactions as polyphenols, sugars, proteins and vitamins will help us better define this relationship. Antiradical activity of cladode juice was between 1.78±0.03 – 4.10±0.02 µmol TE/mL by DPPH assay. It was reported that antioxidant activity of lemon juice by DPPH assay reached 0.26 ± 0.09 µmol TE/mL, it is far lower compared to juice cladode [6]. Thus, even if we had a slow reaction which can be due to steric inaccessibility or proteins involving polyphenols, the richness of the juice in antioxidant gives us great values.

4 CONCLUSION

From the presented data, cladode juice has been subject to several analyses due to its great compositional diversity. Proximate composition showed that cladode juice is rich in potassium and ascorbic acid but poor in sugars which make cladodes a low-calorie food. Cladode juice extracted in august gave the highest values for most analyses (proximate composition, polyphenols content, and other compounds). Warm periods minimize water retention so cladodes have a high concentration of bimolecular compounds. It was also noticed that spiny cladodes has a higher antioxidant activity and polyphenol content than the spineless species. The present work showed that the juice cladodes serve as a good source of natural antioxidant compounds suitable for application in the pharmaceutical field and could potentially be considered as a functional food or functional food ingredient. Nowadays, this hidden knowledge needs to be discovered and re-evaluated. Sophisticated analytical approaches and innovative processing technologies will open new avenues to further promote the use of cactus pear stems, fruits and flowers in food, medicine, cosmetic, and pharmaceutical industries. An increasing demand would help encourage farmers to increase their acreage and thus also help to counterbalance erosion.

References

[1] M. Abid, S. Jabbar, T. Wu, M. Muhammad Hashim, B. Hu, S. Lei, and X. Zeng, “Sonication enhances polyphenolic compounds, sugars, carotenoids and mineral elements of apple juice,” Ultrasonics Sonochemistry, 21, pp. 93-97, 2014.

[2] S. Akhtar, F. Khan, and B. Javid, “Nutritional Composition, Sensory Evaluation and Quality Assessment of Different Brands of Commercial Tetra Pack Apple Juices Available in Local Market of Peshawar Pakistan”, Global Journal of Biotech. and Biochem., 8, pp. 69-73, 2013.

[3] M. J. T. J. Arts, G. R. M. M. Haenen, L. C. Wilms, S. A. J. N. Beetstra, C. G. M. Heijnen, H. P. Voss, and A. Bast, “Interactions between flavonoids and proteins: Effect on the total antioxidant capacity”, J. Agric. Food Chem., 50, pp. 1184-1187, 2001.

[4] N. Balasundram, K. Sundram, and Samman S, “Phenolic compounds in plants and agri-industrial by-products: Antioxidant activity, occurrence, and potential uses”, Food Chemistry, 99, pp. 191–203, 2006.

[5] N. Barka, K. Ouzaouit, M. Abdennouri, and M. El Makhfouk, “Dried prickly pear cactus (Opuntia ficus indica) cladodes as a low-cost and eco-friendly biosorbent for dyes removal from aqueous solutions”, Journal of the Taiwan Institute of Chemical Engineers, 44, pp. 52–60, 2013

[6] D. Barreca, E. Bellocco, C. Caristi, U. Leuzzi, and G. Gattuso, “Flavonoid profile and radical-scavenging activity of Mediterranean sweet lemon (Citrus limetta Risso) juice”, Food Chemistry, 129, pp. 417–422, 2011.

[7] W. Brand-Williams, E. Cuvelier, and C. Berset,” Use of a free Radical Method to Evaluate Antioxidant Activity”, Lebensm. Wiss. Technol., 28, pp. 25-30, 1994.

[8] V. Braescoa, T. Gauthier, and F. Bellisle, “Fruit juices and fruit nectars”, Cahiers de nutrition et de diététique, 48, pp. 248-256, 2013.

[9] N. Chougui, H. Louaileche, S. Mohedeb, Y. Mouloudj, Y. Hammaoui, and A. Tamendjari, “Physico-chemical characterization and antioxidant activity of some Opuntia ficus-indica varieties grown in North Algeria”, Afr. J. Biotechnol., 12, pp. 299-307, 2013.

[10] M.S. Defelice, “Prickly pear Cactus, Opuntia spp.,A Spine-Tingling, Tale”, Weed. Technol., 18, pp. 869-877, 2004.

[11] M. Dos Santos Lima, I. De Souza Veras Silani, Maia Toaldo, L. Claudio Corrêa, L. Camarão Telles Biasoto, G. Elias Pereira, and M.T. Bordignon-Luiz, “Phenolic compounds, organic acids and

antioxidant activity of grape juices produced from new Brazilian varieties planted in the Northeast Region of Brazil", Food Chem., 161, pp. 94-103 , 2014.

[12] M. Dubois, K. Gilles, J.K. Hamilton, P.A. Rebers, and F. Smith, "Colorimetric method for determination of sugars and related substances", Anal. Chem., 28, pp. 350-356, 1965.

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

[14] A.H. El-Sayed, M.A. Nagaty, M.S. Salman, and S.A. Bazaid, "Phytochemicals, nutritionals and antioxidant properties of two prickly pear cactus cultivars (Opuntia ficus indica Mill.) growing in Taif", Food. Chem., 160, pp. 31-38, 2014.

[15] G. Giovanna, M.L. Parker, R.N. Bennett, J. Robertson, G. Mandalari, A. Narbard, R.B. Lo Curto, G. Bisignano, C.B. Faulds, and K.W. Waldron, K.W, "Anatomical, chemical, and biochemical Characterization of cladodes from Prickly Pear (Opuntia ficus-indica (L.) Mill.)" J. Agric. Food. Chem., 57, pp. 10323-10330, 2009.

[16] G. Goldstein, L. Andrade, and P. Nobel, "Differences in Water Relations Parameters from the Chlorenchyma and the Parenchyma of the Opuntia ficus-indica under Wet versus Dry Conditions" Aust. J. Plant. Physiol, 18, pp. 95-107, 1991.

[17] J.M.A. Joslyn, "Tannins and related Phenolics". In Methods in food analysis, p. 701-725, 1970.

[18] H. Kelebek, A. Canbas, and S. Selli, "Determination of phenolic composition and antioxidant capacity of blood orange juices obtained from cvs. Moro and Sanguinello (Citrus sinensis (L.) Osbeck) grown in Turkey", Food Chemistry, 107: 1710–1716, 2008.

[19] A. Konic-Ristic, K. Savikinb, G. Zdunic, T. Jankovic, Z. Juranicc, N. Menkovic, and I. Stankovic, , "Biological activity and chemical composition of different berry juices", Food Chemistry, 125, pp. 1412–1417, 2011.

[20] J. Kroll, H. M. Rawel, and S. Rohn, "Reactions of plant phenolics with food proteins and enzymes under special consideration of covalent bonds", Food Sci. Technol. Res., 9, pp. 205-218, 2003.

[21] J.L. Lamaison, and A. Carnat, A., "Teneurs en principaux flavonoids des fleurs de Crataegeus monogyna Jacq et de Crataegeus Laevigita (Poiret D.C) en fonction de la vegetation", Pharm. Acta helv., 65, pp. 315-320, 1990.

[22] H.N. Le Houérou, "Cacti (Opuntia spp.) as a fodder crop for marginal lands in the Mediterranean basin. Proc. 4th IC on Cactus pear and cochineal", Acta Hort., 581, pp. 21–46, 2002.

[23] M.A. Livrea, and L. Tesoriere, L., "Health benefits and bioactive components of the fruitsfrom Opuntia ficus -indica [L.] Mill", Journal of the Professional Association for Cactus Development, 8, pp. 73–90, 2006.

[24] O.H. Lowry, and N.J. Rosenbrough., "Farr A.L., Randall R.J. Protein measurement with the Folin Phenol reagent", J. Biol. Chem., 193, pp. 2665-275, 1951.

[25] Y. Mohamed-Yasseen, S.A. Barringer, and W.E. Splittstoesser, "A note on the uses of Opuntia spp. In Central/North America", J. Agric. Environ., 32, pp. 347-353, 1996.

[26] N. Muhacir-Güzel, M. Türkyilmaz, O. Yemis, S. Tagi, and M. Ozkan, "Changes in hydrolysable and condensed tannins of pomegranate (Punica granatum L., cv. Hicaznar) juices from sacs and whole fruits during production and their relation with antioxidant activity", Food Science and Technology, 59, pp. 933-940, 2014.

[27] M. Pinent, A. Castell, I. Baiges, G. Montagut , L. Arola, and A. Ardévol, "Bioactivity of flavonoids on insulin-secreting cells", Compr. Rev. Food. Sci., 7, pp. 299-308, 2008.

[28] K.W. Prasad, Kong, K.W., N.S. Ramannan, A. Azrina, and I. Amin, "Selection of experimental domain using two level factorial design to determine extract yield, antioxidant capacity, phenolics and flavonoids from Mangifera pajang Kosterm", Sep. Sci. Techno., 47, pp. 2417-2423, 2012.

[29] S.0. Renaud, and M. De Lorgeril, "Wine, alcohol, platelets and the French paradox for coronary heart disease", Lancet, 339, pp. 1523-1526, 1992

[30] N. Retamal, J.M. Duran, and J. Fernandez, "Seasonal variations of chemical composition in prickly pear (Opuntia ficus- indica(L.)Miller)", J. Sci. Food. Agric., 38, pp. 303-311, 1987.

[31] C.A. Rice-Evans, N.J. Miller, and G. Paganga, "Structure-antioxidant activity relationships of flavonoids and phenolic acids", Free Radical Bio. Med., 20, pp. 933-956, 1995.

[32] A. Rodriguez-Felix, and M. Cantwell, "Development and changes in composition and quality of prickly pear cactus cladodes (nopalitos)", Plant. Foods. Hum. Nutr., 38, pp. 83-93, 1988.

[33] S.M. Rohn, H. Rawel, H., J. Kroll, “Antioxidant Activity of Protein-Bound Quercetin”. J. Agric. Food Chem., 52, pp. 4725−4729, 2004.

[34] V.L. Singleton, R. Orthofer, and R.M. Lamuela., “Analyses of total phenols and other oxidation substances and antioxidants by means of folin-Ciocalteu reagent. Oxidant and Antioxidants Part A”, 299, pp. 152-178, 1999.

[35] F.C. Stintzing, K.M. Herbach, M.R. Mosshammer, R. Carle, W. Yi, and S. Sellappan, “Color, betalain pattern, and antioxidant properties of cactus pear (Opuntia spp.) clones”, Journal of Agricultural and Food Chemistry, 53, pp. 442–451, 2005.

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

[37] H.A. Synman, “ A greenhouse study on root dynamics of cactus pears, Opuntia ficus-indica and O. robusta”, J. Arid. Environ., 65, pp. 529-542, 2006.

[38] Y.S. Velioglu, G. Mazza, L. Gao, and B.D. Oomah, “Antioxidant activity and total phenolics in selected fruits, vegetables, and grain products”, J. Agric. Food. Chem., 46, pp. 4113-4117, 1998.

[39] R.B. Willis, and P.R.N. Allen, 1998, “Improved method for measuring hydrolysable tannins using potassium iodate”, The analyst., 123, 435-439.

[40] G. Xu, D. Liu, J. Chen, X. Ye, Y. Ma, and J. Shi, “Juice components and antioxidant capacity of citrus varieties cultivated in China. Food Chemistry”, 106, pp. 545–551, 2008.