Phytochemical Constituents And In Vitro Radical Scavenging Activity Of Different Cladodes Juice Of Cactacea Cultivars From Different Areas In Morocco

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ABSTRACT: The antioxidant activities of prickly pear cladode juice were studied by DPPH, TEAC and FRAP methods. The chemical composition of the samples was determined by liquid chromatography. The antioxidant properties of different cladode juice for different regions were studied. The region of EL Kelaa registered the highest values in antioxidant activities and total polyphenols: 13.19 mmol Fe^{2+}/L, 70.21 mg Trolox/L, 4654.83 mg Trolox/L were done by FRAP, DPPH and TEAC assays respectively and 477, 95 mg Gallic acid / L in polyphenols content. Spiny cladodes had higher values for most analyses compared to spineless cladodes. A correlation between polyphenols, flavonoids and antioxidant activity was observed, which indicates the implication of those components in antioxidant power. Results of the present study confirmed that bimolecular compounds of cladode juice can be used in pharmaceutical and cosmetic applications.

Keywords: Cladode, Prickly, cactacee, diatery fiber, Antioxidant activity, polyphenols, flavonoids, DPPH, FRAP, TEAC.

1. INTRODUCTION

The prickly pear cactus is an endemic plant of America. There are more than 258 known species and 114 of them can be found in Mexico. In Morocco, several species from Opuntia genera have been found. These succulent perennial plants like trees reach 3–5 m with a woody trunk and stems 30–60 cm long or more. The stems are formed by groups of opaque-green cladodes, with areolas that contain numerous thorns; they produce large yellow flowers, followed by sweet yellow or reddish-purple fruits. The two best-known species of this Moroccan cactus is Opuntia ficus-indica (spineless) and Opuntia megacantha (spiny). For centuries, cladodes have been a nutritional source in Mexico. Mexican diet includes cladodes cooked as vegetables and combines them in a variety of salads, soups, stews, and other meals[1]. The cactus pear fruit of Opuntia ficus-indica is associated with the semi-arid zones of the world; it is one of the few crops that can be cultivated in areas which offer a very little growth possibility for common fruits and vegetables [2]. The prickly pear cactus (Opuntia ficus indica; Opuntia spp.,Cactaceae) is native to the United States, Mexico and South America, but it grows well in other areas, including Africa, Australia and the Mediterranean region [3]. Recently, in some countries such as Mexico, Italy and South Africa, the prickly pear is grown on large surfaces for industrial purposes. In Mexico, his culture extends over an area of 300,000 ha, while in Morocco, the area of Prickly Pear cultivation is about 120,000 ha [4,5]. With the exception of the Sahara and mountainous regions, the prickly pear is widely present in the Moroccan countryside, but its cultivation is poorly organized and structured. In Morocco, the cultivation of prickly pear is made for traditional uses such as boundary hedges, fight against erosion, improvement of pastoral areas or for fresh fruit consumption. The major components of the fruit pulp are 85% of water, 10–15% of carbohydrates, and substantial amounts of vitamin C 0.025–0.030% [6]. Its nutritional value lies essentially in its glucose and fructose content (6–8%) [7]. The level of ascorbic acid is moderate (0.023%) and the acidity is low (0.06%). Prickly pear cladode can be used in many ways in diverse sectors, utilizing different parts of the plant like the food sector and rich food. The chemical and mineral composition described by different author's shows that cactus pears have a similar nutritive value to other fruits. However, its soluble solids content reaches values greater than 16% compared to other fruits, such as prune, apricot, and peach [8,9,10]. The other components present in cactus pear pulp are protein (0.21–1.6%), fat (0.09–0.7%), dietary fiber (0.02–3.15%) and ash (0.4–1%), all of them are similar values to other fruits [11,12,13,14]. The total content of free amino acids is 257.24 mg /100 g, this value was only found in citrus and grape, and it is above average in other fruits. Also characteristic of prickly pear, in comparison with other fruits, is their high content of serine, c-amino butyric acid, glutamine, proline, arginine and histidine, and the presence of methionine [12]. The fruit has a range of colors varying from green to orange to red to purple, and this is an attractive quality parameter for consumers. Colors such as those are produced by the presence of the pigments like chlorophyll and betalain in the green and purple fruits respectively, it is certainly that this parameter makes the fruit and its products attractive; nevertheless, their stability in prickly pear products is still being studied .[15][16][17] Several researchers have quantified and identified betalaines pigments on Mexican or Spanish prickly pears [18][19][20]. Several studies show that cactus pear is a plant rich in vitamins, minerals, amino acids and sugars. It is used as food for his nutritional capacity, medical, cosmetic and production of cochineal. The cactus pear has been and continues to be made in an attempt to better use this species, which has been considered attractive for centuries [21][22] Other studies have shown that the cladode juice is rich in polyphenols. Polyphenols have been studied for their protective effects against pathogenic bacteria and viruses infecting plants or UV radiation. Such as antioxidants, polyphenols have the ability to trap free radicals generated continuously by the body or formed in response to attacks on our environment (smoking, pollution, infections, etc.) [23]. The fruit production of the prickly pear (Opuntia ficus-indica) was a long marginalized, but now it is on increase, given a socio-

economic and environmental importance of this culture. These fruits are rich in vitamin C, betalaines (pigments), phenolic compounds, reducing sugars and minerals [20][24]. In this context, the objective of this work is the identification of some polyphenols which can be present in the juice and cladodes of different cultivars in different Moroccan regions and the evaluation of their antioxidant powers. Actually in Tunisia and in other Mediterranean countries, cactus pear plant grows spontaneously and consumed exclusively as fresh fruit and cladodes as an animal feed complement. Only a small quantity is being used for processing; so, there is need to create a better outlet for seasonally surplus production which used in animal feeds or otherwise go to waste. The actual trend to find new sources of dietary fiber and natural antioxidant such as agronomic by-products that have traditionally been undervalued was more important. In this context cladodes from Opuntia ficus indica f. plant could be a great source of bio-molecule such as dietary fiber, mineral and natural antioxidant compounds. In the literature the available data have been especially concerned general physicochemical composition and processing of pulp and seeds [25,26]. The culture of Opuntia exists almost in all regions of the country with relatively variable area; it occupies an estimated area with about 54,530 ha, representing 11.07% of the total area of the fruit trees [4]. Its geographical distribution is quite large since it found both in coastal areas from Sidi Ifni in the South to Tangier in the North, as in several continental regions [4]. The best plantations are located in coastal areas and more specifically in the coastal strip of more than 10 km wide undergoing maritime influence, the plant benefits the night and morning fog, very frequently in this area throughout year [4][27]. The cactus has been largely ignored by scientists until the beginning of 1980; this renewed interest is partly attributed to the multifunctionality of prickly pear fruit. Recent studies have revealed their high levels of certain chemical compounds, which can make this fruit an added nutritional value, such as taurine, calcium, magnesium, phenolic compounds and betalains [28][29] In Morocco, the new strategy of agricultural development concretized by the Green Morocco Plan (PMV) is a real opportunity for the development of different sectors related to cactus, knowing that the PMV provides the consecration of a million hectares fruit species requiring little water, such as olive, carob or cactus [27]. The area reserved for the latter in each region will experience a remarkable increase, especially in the Guelmim-Smara and Rhamna regions with additional 70,000 and 50,000 ha respectively. Moroccan cactus has a very high genetic variability, several cultivars exist [27], and are distinguished by the flowering period (early, late), the flower color (yellow, orange and pink), fruit color and pulp (green, yellow, orange, red and purple), fruit shape (oval, round or oblong), the organoleptic characteristics of fruit [4] and the antioxidant content[30][31] The fruits of the prickly pear exhibit both intra-site and inter-site variability in the shape, color, weight, sugar, acids, antioxidants, etc. These parameters vary from one cultivar to another and are strongly influenced by the environment [32]. In this context, we are interested in the study of antioxidants (polyphenols, flavonoids and cladode juice of both cultivars (spinly and spinelss) in the regions of tadla Azilal and ouardigha.

2. Material and Methods

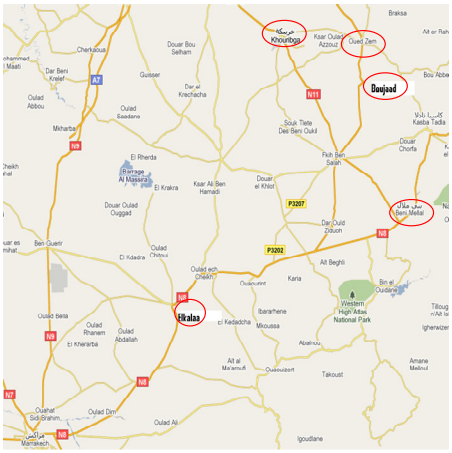
2.1. Plant Material

The cactus pear cladodes were collected in July (2012) in different regions (Kouribga, Beni mellal, Bejaad, Oued Zam, and Kelaa) in central Morocco (Fig.1). They were repeatedly washed with distilled water to remove dirt particles and they were cut into cubes of 2 cm.

Sampling Sites

The fruit samples were collected from five different sites in their relief and climate; Tadla Azilal, Chaouia Ourdigha had a maritime influence, located at 100 km from the sea and 200 km southwest of the Marrakech city and Asgherkis a continental site located at 709 m of altitude, and 57 km in the south of the Beni mellal City (70 km from khouribga) (Fig. 1).

Fig. 1: *Sampling regions of cactus pear cladodes*



The samples of this study are 3 linked cladodes (R1, R2 and R3), differing in age and height (Fig.2). Cladodes were washed and peeled. Pulp and juice were separated by centrifugation. The juice obtained was pasteurized and stored at -20 ° C until analysis.

***Fig.2**: Linked Cladodes of cactus pear*

2.2. CHEMICAL ANALYSIS

Moisture , total ash, Titrable acidity, and degree Brix were determined according to methods AOAC [33]; The sugar content of the juice was determined using a refractometer (DIGIT 032), the results are expressed as Brix. The free acidity, expressed as a percentage of citric acid is determined by titration; 10 ml of the juice of prickly pear was placed in a 100 ml beaker equipped with a magnetic stirrer, 20 ml of distilled water was added and the homogenized mixture is titrated with NaOH (0.1 N) to pH = 8.1 [34]; The proximate analyses of the samples for moisture AOAC [33]crude fat and total ash were carried out in triplicate

POMOLOGICAL CHARACTERISTIC

In this research work, forty morphological traits were investigated and analyzed including plant weight, length , ratio L/W, width of cladode for tree position (R1, R2, R3)

ASHES

Samples weighing approximately 10 g of each dried fruit cladode were placed in previously weighed porcelain crucibles. The samples were then carbonised over a Bunsen burner and placed in a muffle furnace where they were heated to 550 C, left at this temperature for 2 h and then transferred to a desiccators containing silica gel. After reaching room temperature, the crucibles containing the samples were weighed to determine the ash content by difference. Again, there were tree repetitions (n =3).

SOLUBLE AND INSOLUBLE DIETARY FIBER DETERMINATION: CRUDE FIBER: DIETARY FIBER

The dietary fiber estimation was done by an enzymatic gravimetric method Asp [35] with some modifications [36]. To 5 mL of juice 20 mL of sodium phosphate buffer (pH 6.0) was added and homogenized, followed by the addition of alpha-amylase, protease and amyloglucosidase. After a filtration, the residue and filtrate have been used to determine insoluble and soluble fiber respectively. The residue was washed with water, then with acetone. It was then dried in oven at 40℃ for 1hour and 105℃ for 30 min and weight. The filtrate was precipitated in ethanol followed by a filtration, centrifugation and washed with ethanol and then with acetone. It was then dried in oven at 40℃ for 1hour and 105℃ for 30 min and weight.

2.3. POLYPHENOLS CONTENT

Total polyphenols content was determined by the Folin-Ciocalteu reagent according to the method [37]. The amount of total polyphenols was measured at 750 nm by spectrophotometry (S2100 Diode Array) and it was calculated as equivalent Gallic acid which is used as standard at a concentration of 0.1mg/mL [38]. A dilution of 1/10 was made followed by a centrifugation 8000tr / min for 7min at 4 ℃, and 0.5 mL of the juice was mixed with 2.4 mL of a solution of methanol and demineralized water (50:50), 2 mL of sodium carbonate 2% (Na2CO3), and 0.1 mL of Folin-Ciocalteau reagent. After incubation at room temperature (25 ℃) for 60 min, the absorbance was measured at 750 nm (S2100 Diode Array). The concentration of total polyphenols is expressed as Gallic acid per 100ml of juice.

2.4. TOTAL FLAVONOIDS

Total flavonoids were done by the method of Dowd as it was adapted by [39]. Flavonoids composition was calculated by spectrophotometry. This method is based on the formation of a complex flavonoid-aluminum, with a maximum absorbance at 430 nm. Rutin was used for the calibration range. Thus, 1 mL of the juice cladode was diluted at 1/20 with MeOH-water juice (1:1), and mixed with 1 mL of a methanolic solution (2% aluminum chloride). After incubation for 15 minutes at room temperature, the absorbance was measured at 430nm, and the results are expressed in mg/g of rutin.

2.5. IDENTIFICATION OF POLYPHENOLS BY HPLC

The identification of polyphenols on juice cladodes was determined by HPLC analysis using a system (1100 series HPLC system with diode array detector) coupled to a computer (HP ChemStation) and a thermostat control. Phenolic compounds in the samples were quantified using standard curves of standard solutions injected into the HPLC. The analysis conditions are made with a C18 column Nucleodur (particle size 5 microns, L = 250 mm, d = 4, 6 mm) maintained at 30 ℃. The flow rate was 1.0 mL / min and the injection volume was 5 µL. The samples were thermostated at 15 ℃ to avoid their degradation. The eluent type used was: Solvent A: 95% v / v water HPLC quality and 5% v / v formic acid; Solvent B: 80% v / v / v acetonitrile, 15% v / v / v water quality HPLC and 5% v / v / v formic acid.

2.6. ANTIOXIDANT ACTIVITY ASSESSMENTS

2.6.1 DETERMINATION OF THE FREE DPPH RADICAL SCAVENGING EFFECT

The DPPH assay was done according to the method of [40] prepared with some modifications. It involves measuring the ability of the extract to trap the free radical DPPH•. To 100 µL of diluted juice with methanol-water (1/1), 2 mL of the methanol solution of DPPH 0.1 mol/L was added. After incubation for 30 minutes at room temperature (25 ℃) protected from light, the absorbance was measured at 517nm. From the absorbance measurements, the percentage of inhibition of DPPH is calculated. This percentage is plotted as a function of the concentration of polyphenol to obtain EC50 (amount of antioxidant required to reduce the absorbance of 50%). The results are

expressed relative to Trolox. DPPH• radical scavenging capacity was calculated using following equation: Scavenging activity (%) = (Absorbance of Blank - Absorbance of Sample / absorbance of the blank) x 100.

2.6.2. ANTIOXIDANT CAPACITY DETERMINATION BY FRAP ASSAY

The FRAP (Ferric Reducing Ability of Plasma) assay was done according to Benzie and Strain (1996) measured by FRAP reagent. 40µL of the juice was diluted with MeOH-water (1: 1) to which is added 0.2 mL of distilled water and 1.8 mL of reagent FRAP. After incubation at 37 °C for 10 min, the absorbance was measured at 593 nm. To evaluate the reduction of iron, a calibration curve was developed by different concentrations of FeSO4, 7H2O (300-1100µmol). The results are expressed in mmol Fe 2+ / L.

2.6.3. ANTIOXIDANT CAPACITY DETERMINATION BY TEAC ASSAY

The antioxidant capacity method according to TEAC (Trolox Equivalent Antioxidant Capacity) test or ABTS was done by the method [41]. 7 mM of ABTS•+ (2,2'-azinobis (3-ethylbenzothiazoline-6-sulphonic acid)) was made in demineralized water and 2.45 mM solution of potassium, they were mixed with a ratio of 1: 0.5. The mixture was allowed to stand protected from the light at room temperature for 12 to 16 hours. The solution was diluted with ethanol to get an absorption of 0.700 (± 0.020) at 734 nm. The working solution was prepared by mixing 1.0 mL of diluted ABTS•+ solution with 10 µL of the cladode extract and the absorbance was measured after 30 min of incubation at 734nm. The antioxidant capacity was expressed as Trolox equivalent as µM or mM Trolox equivalent per gram or per milliliter.

3. RESULTS AND DISCUSSION

3.1. MORPHOLOGICAL AND CHEMICAL COMPOSITION

The result of The cladode weight, width, ratio L/W and length from different areas were summarized in the Table.1. Most of the data points are grouped at the bottom, that is, at low fruit weight for the Tadla azilal city (247757g) and the higher weight (Table.1). While the Higher length founded for Tadla azilal city (25-34cm) and 1,8-2,08 for L/W the lower cladode is for Haouz city with 14-19,5cm for length and the ratio was 1,50-1,77 (Table.1). In addition, intercomparizon of the spiny and spinless cladode (Khouribga) it was noted that for the spiny the ratio L/W is lower (1,42-1,78) than the spinless cladode (1,73-1,94). Table 1 summarized the general composition of spiny and spineless cladodes juice from different region and position. The results showed that the juice content in cladodes varies depending on the regions ranging from 19.75 to 69.68 mL/100g (Table.1). The pH was intermediate (4.51-5.83). The acidity was low and was around 0.40 Equivalent citric acid (g/L). It can be explained by the presence of many organic acids as malic, citric and oxalic acids [42] as salts form. The juice had higher percentages of crude fiber, total ash. Indeed, [43] reported a pH value of 4.6 for cladodes of O. ficus indica f. amylocea and O. ficus indica f. inermis cultivated in Mexico. Water activity of fresh cladodes was about 0.76 for the two varieties. pH and aw value of cladodes could prevent bacteria development but could not prevent moulds and yeast development [44]. Ash contents observed in this study were higher than value reported by [43] and [45] and then higher of Aloe leafs (15,48%) (A. Gulia, 2010)[46] The two varieties of cladodes exhibit a low pH value. This low pH could be explained by the presence of many organic acids as malic, citric and oxalic acids [42]. Indeed, [43] reported a pH value of 4.6 for cladodes of O. ficus indica f. amylocea and O. ficus indica f. inermis cultivated in Mexico. Water activity of fresh cladodes was about 0.76 for the two varieties. pH and aw value of cladodes could prevent bacteria development but could not prevent moulds and yeast development [44]. At the Ouardigha site, the average pH of the cladode juice was 4,62-5,51 for the Khouribga , Ouedzam and Boujaad population and 4,68-5.58 for the Kelaa one, 4,65-4,75 for Beni Mellal one. The citric acid values comprised between 0.32g/l and 0,42 g/l were significantly different (Table 1), these results are with agreement with data reported in the literature (0.05–0.18).

TABLE 1: *PHYSICOCHEMICAL CHARACTERIZATION OF CLADODE JUICE OF PRICKLY PEAR FROM DIFFERENT LOCALITIES*

Regions	Cultivars	Cladodes position	Length (cm)	Width (cm)	Ratio L/W	Weight (g)	Juice content ml/100g	pH	Titrable acidity Equivalent citric acid (g/L)	Ash (g/100g of dry matter)	Degrees Brix (°Bx)
Khouribga A	Spiny	R3	38.50	23.00	1.67	2683.20	19.75	4.62±0.015	0.42±0.01	25.43	3.7
		R2	32.00	18.00	1.78	1443.30	30.49	4.45±0.011	0.41±0.02	25.23	4.5
		R1	22.00	15.50	1.42	710.40	52.79	4.71±0.03	0.41±0.015	25.16	4.4
	Spineless	R3	32.00	18.50	1.73	2265.70	39.06	4.48±0.026	0.38±0.011	22.21	4.5
		R2	30.00	17.00	1.76	1213.50	32.55	5.15±0.011	0.39±0.011	22.12	4.2
		R1	30.00	15.50	1.94	863.40	31.27	4.61±0.015	0.38±0.01	22.01	4.3
OuadZem A	Spineless	R3	42.00	23.00	1.83	2299.30	58.50	5.2±0.05	0.36±0.03	21.32	5.7
		R2	30.00	17.50	1.71	968.50	62.98	5.51±0.026	0.37±0.012	21.22	6,00
		R1	27.00	14.00	1.93	559.70	69.68	4.98±0.08	0.34±0.02	21.13	5.3
Boujaad A	Spineless	R3	39.00	20.00	1.95	1176.70	50.99	5.2±0.1	0.37±0.013	20.25	3.9
		R2	30.5	19.50	1.56	874.20	52.62	5.83±0.01	0.37±0.013	20.76	4.3
		R1	33.00	17.50	1.89	610.90	49.11	5.77±0.01	0.33±0.03	20.23	4.5
Elkalaa B	Spineless	R 3	34.50	19.50	1.77	1717.7	39.59	4.68±0.02	0.36±0.04	21.27	5.8
		R2	32.14	18.50	1.74	1151.20	52.12	5.21±0.026	0.36±0.012	21.22	6.1
		R1	21.20	14,00	1.51	399.80	68.78	5.38±0.021	0.37±0.013	21.26	5.9
Beni Mellal C	Spineless	R3	34.00	16.50	2.06	757.60	60.45	4.94±0.005	0.36±0.02	21.29	3.2
		R2	27.00	15.00	1.80	603.00	43.12	4.65±0.017	0.32±0.021	21.28	3.4
		R1	25.00	12.00	2.08	247.00	56.68	4.75±0.025	0.35±0.013	21.22	3.2

ZONE A: OUARDIGHA, ZONE B: HAOUZ, ZONE C: TADLA AZILAL LOCALITIES

SUGAR CONTENT

For the Haouz site the Kelaa population cladode juice have an average sugar content of 5,9-6,1 Brix higher than other population from other site (Table.1). The lower content were enregistred for chaouiya ouardigha and Tadla Azilal with Beni Mellal, Kouribga and Boujaad population (3,2; 3,7 and 4,2) respectively. On the other hand, the intra-site comparison (for spiny and spineles) showed that the average sugar content of the two cultivars was statistically different for the Khouribga spineless Ouardigha site, while analysis of variance showed a significant difference between these two cultivars. Total dietary fiber, soluble dietary fiber and insoluble dietary fiber were calculates according to the method described by Prosky et al. (1988)[47]. The content of dietary fibers was higher for spiny cladodes compared to spineless cladodes (Table.2). The total fibers varies depending of regions with respectively FAT (Khouribga: 58,2%) for spinless and spinly cultivar (66,18), FAT (Ouad-Zem:58,62%) for spinless, FAT (Beni Mellal:54,90%) , FAT (Boujaad:53,86%) and FAT (Elkalaa:53,62%). Spiny cladodes of the different site were richer in FAT than spineless cladodes. These result were higher than crude of Aloe leaf [46] and FAT content of those reported by M.A. Ayadi et al [48] for Tunisian cladodes and Sàenz [49] for Mexican cladodes. For the two Tunisian varieties of cladodes, FAI amount was higher than FAS amount. The ratio of FAS/FAI was 1:3 was similar that the value as reported by [48] but lower that the value reported by [49] while the FAS/FAI ratio is 1:2 in Mexican cladodes.

TABLE. 2: *AMOUNT OF SOLUBLE (FAS), INSOLUBLE (FAI) AND TOTAL FIBER (FAT) FOR THE SPINLY AND SPINLESS CLADODES CULTIVAR FROM DIFFERENT LOCALITIES*

Cultivars	Cladode position	Dietary insoluble fiber (FAI) %	Dietary soluble fiber (FAS)%	Dietary total fiber (FAT)%	FAS/FAI
Khouribga zone A	spinly	49,71±0,84	16,46±0,14	66,18±0,7	0,33
	Spinless	45,45±0,77	13,17±0,04	58,62±0,81	0,29
Oued Zam zone A	Spinless	43,33±0,24	12,30±0,06	55,63±0,18	0,28
Boujaad zone A	Spinless	41,44±0,01	12,42±0,13	53,86±0,12	0,30
Elkalaa zone B	Spinless	41,33±0,37	12,29±0,1	53,62±0,27	0,297
Beni Mellal zone C	Spinless	42,49±0,33	12,42±0,004	54,90±0,33	0,292

ZONE A: OUARDIGHA, ZONE B: HAOUZ, ZONE C: TADLA AZILAL LOCALITIES

3.2. POLYPHENOLS CONTENT AND ANTIOXIDANT ACTIVITY

Phenolic compounds, also called polyphenols, are metabolic products widely distributed in plant foods; they have many biological and pharmacological properties that could provide protection against chronic disease. These compounds have more antioxidant effect than vitamins, they are able to neutralize the effects of oxidative free radicals [50]. Analysis results shown in Table.3 indicate that the concentration of polyphenols varies depending on the nature of the position of cladodes (R1, R2 or R3). According to the exposure and geographical location of cladodes, the concentration of polyphenols varies. It gradually decreases from R1 to R3. Polyphenols content in cladode juice of the cultivar localized in Haouz site with Elkalaa cultivar reached 477.95 ± 0.12 mg Gallic acid/L (R1), it is the highest value compared to other cultivars: 269,11 ± 0.10 mg Gallic acid /L (Khouribga), 269,11 ± 0.10 mg Gallic acid/L (Oued Zem), 263,84 ± 0.13 Gallic acid/L (Boujaad), and 253,19 ± 0.08 mg Gallic acid/L (Beni Mellal). A significant difference in polyphenols content was registered between the spiny and spineless cultivars in the two site Tadla azilal and Chaouia ouardigha. Indeed, the spiny cladodes of Khouribga contain a higher amount of polyphenols than the spineless variety from the same region. This change in content of polyphenols is related to the physiology of spiny cladodes, allowing them to conserve Ouardigha Sahel cladode fruits have an average content of polyphenols of 273-293lmgGAE/g for Khouribga, 263-269GAEmg/L for Oued zem and 254-263mgGAE/L Boujaad cultivars respectively. While in the Haouz site, this content is in the range of 477.95 ± 0,12mGAEg/L to 458,55±0,30mg GAE/l. However for the Tadla azilal site, this content is in the range 171,12±0,30 to 253.19±0.08mg GAE/l for Beni Mellal cultivar (Table.3). This difference between the tree sites is the result of the effect of a number of factors, the main ones being genetic, precipitation, light, topography, soil type and maturity as reported by Harris and Karmas [51]. The mean content of polyphenols comprised between 289.39 and 447.27mg/g is higher than those indicated by Chavez-Santoscoy [52] who determined the values of 55.4–226.3mg/g of nine varieties of Opuntia. Our results are identical to those found on the peach, plum and nectarine ranging from 91 to 1042lmg/g after thanks to polysaccharides [42].

TABLE 3 *ASSESSEMENT OF POLYPHENOLS CONTENT IN CLADODE JUICE OF OPUNTIA FICUS INDICA*

Regions	Cultivars	Cladodes position	total polyphenols (mg gallic acid / L of juice)	Flavonoids content mg RE/L of juice
Khouribga A	Spiny	R1	396.17 ± 0.49	299.48 ±0.15
		R2	283.76 ± 0.29	91.53 ±0.13
		R3	276.04 ± 0.09	89.56 ±0.70
	Spineless	R1	367.64±0.06	308.63 ±0.60
		R2	251.69±0.08	100.73 ±0.50
		R3	251.76±0.07	123.06 ±0.40
OuadZem A	Spineless	R1	269.11±0.10	201.98 ±0.28
		R2	269.95±0.15	83.35 ±0.33
		R3	249.23±0.14	90.04 ±1.12
Boujaad A	Spineless	R1	263.84±-0.13	144.40 ±0.33
		R2	262.02±0.13	122.22 ±0.15
		R3	254.68±0.08	92.34 ±0.38
Elkalaa B	Spineless	R1	477.95±0.12	282.86 ±0.44
		R2	459.59±0.20	84.56 ±0.53
		R3	458.55±0.30	82.94 ±0.46
Beni Mellal C	Spineless	R1	253.19±0.08	148.99 ±0.36
		R2	227.95±0.13	137.62 ±0.42
		R3	171.12±0.15	80.93 ±0.28

ZONE A: OUARDIGHA, ZONE B: HAOUZ, ZONE C: TADLA AZILAL LOCALITIES

Besides Phenolic acids , Cladode juice contain quite a lot more of flavonoids compounds, being also able to contribute to the antioxidant activity. To give a rough estimation, photometric determination of the flavonoids content was carried out The cladode juice of cactacee is rich in flavonoids (Table 3) compared to cladode juice of different areas and position of cladode, a difference significative was observed with 160,19mg RE/l of juice for Ouardigha site (khouribga 125,12±0,33mg RE/l oued zem ; 119,98mg/l boujaad) and Haouz site(; 150,12mg RE/l for Kelaa) and tadla azilal site (122,51mg RE/l juice cladode) for Beni mellal population. In the oder hand , Similarly, in relation to flavonoids (Table.3), a difference in the concentration was noticed between juice cladode from different position(R1, R2 or R3) and this concentration gradually decreases from R1 to R3 in all cultivars for all site. The antioxidant activity of the MeOH extract fractions of cladode juice cactacee was assessed by measuring their ability to scavenge DPPH radicals. The samples were able to inhibit the activity of DPPH radical. While this antiradical activity depends on the regions, cladodes extract has a very important antioxidant activity. Thus, for the region of ELKELAA, antioxidant activity for cladodes in R1 position is higher than R2 which is higher than R3 (Table.4). The analysis of Table 5 indicates that there is a correlation between polyphenols and antioxidant activity (FRAP), which marks the availability of antioxidant compounds. In addition, a correlation between flavonoids and antioxidant activity (FRAP) was observed, most flavonoids have an antioxidant activity. Similarly, another correlation between polyphenols and flavonoids was observed because the most of polyphenols are flavonoids. The TEAC method is well correlated with FRAP method. It was reported that polyphenols in fruit juice was 642, 28 mg/L, and TEAC 18, 61 mmol/L [42]. However polyphenols in our fruit juice was 171,12 - 396,17mg/l and TEAC 15,37-18,31 mmol Fe2+/L.

TABLE 4 *ANTIOXIDANT CAPACITY OF CLADODE JUICE OF PRICKLY PEAR*

Regions	Cultivars	Cladodes Positions	FRAP (mmol/L F^{2+})	DPPH (mg/L)	TEAC (mg/L)
Khouribga A	Spiny	R1	12.19±0.01	47.38±0.20	4583.20±1.55
		R2	11.05 ±0.01	46.06±0.22	4578.43±1.56
		R3	10.23 ±0.00	44.72±0.25	4541.43±1.60
	Spineless	R1	11.99 ±0.01	44.08±0.21	4491.80±1.58
		R2	10.40 ±0.01	43.99±0.19	4448.15±1.45
		R3	9.53 ±0.02	43.41±0.22	4423.59±1.60
OuadZem A	Spineless	R1	10.19 ±0.01	46.99±0.24	4538.87±1.64
		R2	9.90 ±0.01	45.72±0.21	4514.65±1.52
		R3	9.40± 0.01	44.38±0.21	4506.43±1.45
Boujaad A	Spineless	R1	9.26 ±0.00	46.67±0.23	4087.66±1.26
		R2	8.40± 0.01	46.6±0.20	4013.14±1.25
		R3	8.26 ±0.02	45.73±0.23	3838.35±1.44
Elkalaa B	Spineless	R1	13.19 ±0.01	70.21±0.21	4654.83±1.23
		R2	11.41 ±0.02	65.5±0.24	4650.90±1.66
		R3	9.25 ±0.02	61.8±0.22	4639.31±1.75
Béni Mellal	Spineless	R1	8.25 ±0.01	46.39±0.21	3853.53±1.25
		R2	8.11 ±0.02	41.08±0.21	3847.05±1.22
		R3	6.69 ±0.01	40.51±0.23	3847.39±1.65

ZONE A: OUARDIGHA, ZONE B: HAOUZ, ZONE C: TADLA AZILAL LOCALITIES

A correlation was observed between the content of flavonoids and total polyphenols of our extracts and with antioxidant activity. The majority of the antioxidant properties of these plants are due to their polyphenols. Several studies have shown in the literature that the cactus is rich in polyphenol. The most of those studies are focused on flavonoids and seen their importance to our overall health. Indeed, Other reports indicated that the plants of the Cactaceae family contain flavonol 3-O-glycosides (quercetin, kaempferol, and isorhamnetin), dihydroflavonols, flavonones, and flavanonols [53,54]. These results are correlated with the cactus polyphenols HPLC analysis (Fig.3).

TABLE 5 *COEFFICIENT OF DETERMINATION R^2 BETWEEN THE TOTAL POLYPHENOL CONTENT, FLAVONOIDS AND ANTIOXIDANT ACTIVITY BY THE THREE METHODS (DPPH, TEAC, FRAP).*

	Total polyphenols content (mg GAE/L cladodes juice)	Flavonoids (mg RE/l cladodes juice)	DPPH	TEAC	FRAP
Total Polyphenols content mg GAE/L cladodes juice	1	------	--------	----------	------
Flavonoides (mg RE/l cladodes juice)	0.71	1	----------	----------	----------
DPPH	0.22	0.32	1	----------	----------
TEAC	0.56	0.69	0.60	1	----------
FRAP	0.89	0.86	0.46	0.81	1

3.3. Phenolic acid profiles

The revealed antiradical activity of the extract and fractions of Cladode juice of two cactacee has guided its phytochemical analysis, in order to isolate the compounds that could be related to this bioactivity. Based on the correlation between polyphenolics and antioxidant activity [55][56] we searched for these compounds in the MeOH extract and MeOH/eau fractions of cladode juice of cactacee, by analyzing their TLC polyphenolic profiles in two different mobile phase systems and UV (254 and 365 nm) and NP/PEG/UV, a stain used for flavonoids and phenolic acids. The phenolic acid profiles in cladode juice of differents regions are presented in Table 6. The content of catechin acid is the highest(54,68µg/ml) than other polyphenolic compounds 17,58µg/l for Isoquercitrine ; 15,58µg/m for quercetrine ; 13,37µg/ml for Epicatechin and 12,15µg/ml for rutin. The aqueous methanolic extracts of cladode fruit, HPLC– DAD analyses were carried out (table.6).. The concentrations of flavonols in the present cladodes are comparatively lower (25,52µg/ml for isoquercetrine, 16,57µg/ml for quercetrine) than those found in cladodes from Italy (4.8 mg/g) [57], as well as the cladodes from Mexico (1.1–3.5 mg/g) [58]. While the concentration of acid phenol (17,16 µg/ml for Epicatechin, 15,85µg/ml for catechin and 5,42µg/ml for Gallic acid) (Table.6) and Figure.3. The results obtained here in underline to some extent those obtained [57] and [59] from cactus O. ficus-indica cladodes collected from Italy and Mexico, respectively. It have been [59] detected in cladodes from two different cultivars from O. ficusindica separately; Ginestra et al. (2009)[57] detected them in a whole mix of three different cultivars fromO. ficus-indica cladodes. Both studies illustrated that cactus O. ficus-indica cladodes are characterized with mainly isorhamnetin derivatives. In contrast, Guevara-Figueroa et al. (2010)[58] detected isoquercitrin, isorhamnetin-3-O-glucoside, kaempferol- 3-O-rutinoside, rutin and isorhamnetin-3-O-rutinoside in two commercial and three wild varieties of O. ficus-indica cladodes cultivated in Mexico. Further, they reported that the most abundant flavonols were kaempferol-3-O-rutinoside and isorhamnetin-3-O-rutinoside.

Table.6 *Concentration of phenolic compound in methanolic extracts of cladode juice (µg/ml of cladode juice)*

	MeOH		
Molecules	Time retention	Air	Concentration (µg/ml)
Gallic acid	4.456	3.8	0.14
Catechin	18.08	355.01	54.68
Epicatechin	39.6	101.71	13.37
Hyperoside	55.52	14.55	0.65
Isoquercitrin	56.47	377.6	17.58
Quercitrin	58.7	277.8	15.58
Phlorizin	59.26	57.78	2.9
Rutin	57.41	198.33	12.15
Quercetin	65.21	7.91	0.21

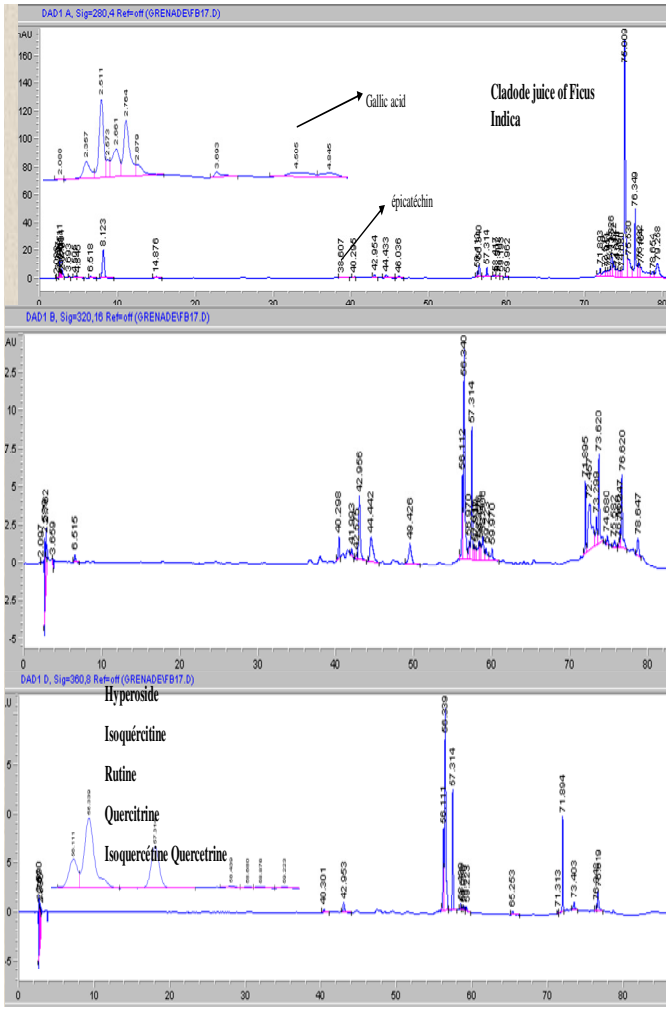


Fig.3 *: Chromatograms of juice cladodes of Opuntia ficus-indica*

The results obtained in the present study showed that kaempferol ,isorhamnetin-3-O-glucoside, kaempferol- 3-O-rutinoside, rutin and isorhamnetin-3-O-rutinoside was not found at all. The concentrations of flavonols in the present cladodes are comparatively higher (6.3–7.3 mg/g DW) than those found in cladodes from Italy (4.8 mg/g)[57], as well as the cladodes from Mexico (1.1–3.5 mg/g) [58]. Further, the level of aglycon reported herein by enzymatic hydrolysis is significantly higher than that reported [59] Studies reported that flavonol formation might be accelerated by increased sun light exposure. For instance, Price, Breen, Valladao, and Watson (1995)[60] investigated the impact of sun exposure level on the concentration of flavonols in wine and reported that quercetin glycosides had 4.5, 14.8, and 33.7 mg/L in wines produced from shaded, moderate, and highly light exposed grapes, respectively. Further, Stewart et al. (2000) [61] investigated the influence of location on the flavonols in twenty different varieties of tomatoes. They reported that the highest concentrations of flavonols were found in tomatoes originating from warm sunny climates in Spain and Israel. Schirrmacher, Schnitzler, and Graßmann (2004)[62] mentioned also that flavonoid contents were higher in field-grown plants than those in greenhouse-grown plants. This might explain the increase in flavonol levels in cactus fruits from South Africa compared to fruits

from Egypt and Sicily. As already mentioned, information about specific cultivation practices was not available, but it was tried to get/collect the full colored fruits at an approximate similar maturity and ripening stage. They also identified two other compounds as Phlorizine and hyperoside derivatives.

4. Conclusion

The physico-chemical study showed that the titrable acidity and water content did not differ significantly between the cultivars cladode of the tree localities studied. Our results also showed that the HPLC determination of total polyphenols shows values between 0,14µg/ml and 58µg/ml of cladode juice. These results indicate that the cladode juice of different areas contain significant levels of polyphenols and specially the acidic phenols and flavonols . This study showed that fruits cladodes are very rich in polyphenols content, flavonoids, and have a remarkable antioxidant activity. The Correlation between polyphenols content and antioxidant activity can be attributed to their phenolic composition. In addition cladodes are an important source of fiber. The spiny cladodes are richer in biomolecular components compared to spineless cladodes. The use of cactus pear stems, fruits and flowers can be done in food, medicine, cosmetic, and pharmaceutical industries. Physico-chemical analysis of spiny (O. ficus indica megantha and Ficus indica). Cladode and spineless cladodes (O. ficus indica f. inermis) obtained from cactus plant growth in Mediterranean area (Morocco) shows a great richness on bio-molecule which could be valorized by including cladodes powders in food formulation to improve nutritional, technological and stability of formulated food stuffs. Fortification of wheat flours by cladodes powders as a source of dietary fiber leads to a change in dough properties. Cladodes like any other fiber source increase water absorption capacity of the flours and consequently the dough properties

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REFERENCES

[1] V.K..Deshpande, A.M. Joshi, Cactus (Opuntia dillenii Grahm) stem: a new source of energy, J. Power ,47,185–188, (1994).

[2] H..Han, P. Felker, Field validation of water-use efficiency of a CAM plant Opuntia ellisiana in south Texas, Journal of Arid Environments, 36, 133–148, (1997).

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

[4] M..Boujnah, L. Chajia, Genetic variation within different Cactus écotypes of Moroccan origin. IVth internat. Congres Cactus Pear and Cochineal and the IVth general Meeting FAO-CACTUSNET. Hammamet, Tunisie, (2000).

[5] M..Arba, Dellahia a cactus pear cultivar from the Mediterranean coast of northern Morocco.Acta horticulturae, 728, 37-41, (2006).

[6] S. Gurrieri, L. Miceli, C. M..Lanza, F. Tomaselli, R. P. Bonomo, & E. Rizzarelli, Chemical Characterization of Sicilian Prickly Pear (Opuntia ficus indica) and Perspectives for the Storage of Its Juice. Journal of Agricultural and Food Chemistry, 48, 5424-5431, (2000).

[7] Y. Habibi, M.. Mahrouz, M..Vignon, Isolation and structure of D-xylans from pericarp seeds of Opuntia ficus-indica prickly pear fruits. Carbohydrate Research, 337, 1593–1598, (2002).

[8] E. Pimienta, El Nopal tunero (1st Edn). Jalisco, MeHxico: Universidad de Guadalajara, 246 pp. (1990).

[9] H..Schmidt-Hebbel, I.. Pennacchiotti, L. Masson, & M.A. Mella, Tabla de composicion quımica de alimentos chilenos (8th Edn). Facultad de Ciencias QumHmicas y FarmaceHuticas, Universidad de Chile. Santiago, Chile. 62 pp, (1990).

[10] E. Sepuhlveda, C. SaHenz, Chemical and physical characteristics of prickly pear (Opuntia ficus indica) pulp. Revista de Agroquimica y Tecnologia de Alimentos, 30, 551–555. (1990).

[11] O.Paredes, R .Rojo, Estudio para el enlatado del jugo de tuna. Tecnologia de Alimentos, 8, 237–240, (1973).

[12] A. Askar, S.K. El-Samahy, Chemical composition of prickly pear fruits. Deutsche Lebensmittel-Rundschau, 77, 279–281, (1981).

[13] W.N. Sawaya, , J.K. .Khalil, M.M. Al-Mohammad, Nutritive value of prickly pear seeds, Opuntia ficus indica. Plant Foods For Human Nutrition, 33, 91–97, (1983).

[14] S. Rodriguez, C. Orphee, S. Macias, S. Generoso, L. Gomes GarcmHa, Tuna:Propiedades fmHsico-qumHmicas de dos variedades. La AlimentacioHn Latinoamericana, 210, 34–37, (1996).

[15] U. Merin, S. Gagel, G.. Popel, S. Bernstein, I. .Rosenthal, Thermal degradation kinetics of prickly-pear-fruit red pigment. Journal of Food Sciences, 52, 485–486, (1987).

[16] D. Montefiori, Ricerche sull’estrazione, la stabilitaH e l’impiego dei pigmenti del fico d’india sanguigno. Tesi di Laurea. UniversitaH degli Studi di Milano, Italia. 94 pp, (1990).

[17] E. Castellanos-Santiago, E.M. Yahia, Identification and quantification of betalains from the fruits of 10 Mexican prickly pear cultivars by high-performance liquid chromatography and electrospray ionization mass

spectrometry. J. Agric. Food Chem., 56, 5758–5764, (2008).

[18] J. Fernandez Lopez, A. Luis, Application of high-performance liquid chromatography to the characterization of the betalain pigments in prickly pear fruits. J. Chromatogr. A, 913, 415–420, (2001).

[19] omar khatabi 2013

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

[21] F.C. Stintzing, A. Schieber, Evaluation of colour properties and chemical quality parameters of cactus juices. European Food Research and Technology, 216, 303-311, (2003).

[22] A., Scalbert, G. Williamson,Dietary intake and bioavailability of polyphenols. J. Nutr., 130, 2073–2085 ,(2000).

[23] B.S..Maataoui, S. Hilali ,Composition physico-chimique de jus de deux types de fruits de figuier de Barbarie (Opuntia ficus indica) cultivés au Maroc Rev. Biol. Biotechnol., 3 (2) , 8–13, ,(2004).

[24] M..Ennouri, E.Bourret, L. Mondolot, H. Attia, Fatty acid composition and rheological behaviour of prickly pear seed oils. Food Chem. 93, 431–437, 2005.

[25] M..Ennouri, H.. Fetoui, E., Bourret, N. Zeghal, H. Attia, Evaluation of some biological parameters of Opuntia ficus indica. 1. Influence of a seed oil supplemented diet on rats. Bioresour. Technol. 97, 1382–1386, 2006.

[26] Z. Bouzoubaaˆ aY. Essoukrati b S. Tahrouch b, A. Hatimi b S. Gharby cH. Harhar, 2014 , Phytochemical study of prickly pear from southern Morocco, Journal of the Saudi Society of Agricultural Sciences (2014) xxx, xxx–xxx

[27] A., Piga, Cactus pear: a fruit of nutraceutical and functionalimportance. J. Prof. Assoc. Cactus Dev. 6, 9–22, 2004.

[28] S. Zine, S. Gharby, M..El Hadek, Physicochemical characterization of Opuntia ficus-indica seed oil from Morocco. Biosci. Biotechnol. Res. Asia 10 (1), 1–7, 2013.

[29] L. Tesoriere, M..Fazzari, M., Allegra, M..Livrea, 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 (20), 7851–7855, 2005.

[30] O. J,.Kuti , Antioxidant compounds from four Opuntia cactus pear fruit varieties. Food chemistry 85(4): 527-533, 2004.

[31] Parish, P.Felker Fruit quality and production of cactus pear (Opuntia spp.). Fruit clones selected for increased frost hardiness J. Arid. Environ., 37 (1) pp. 123–143, (1997),

[32] AACC, Approved Methods of the AACC, 10th ed. American Association of Cereal Chemists, St. Paul, MN (Methods 08-01,30-25,44-15A,46-10,54-10,54-21), 2000.

[33] E.M..Medina, E.M..Rodrı´guez, C.D. Romero, Chemical characterization of Opuntia dillenii and Opuntia ficus indica fruits. Food Chem. 103 (1), 38–45, 2007.

[34] N. G.. Asp, C. G..Johansson,v H.. Hallmer, & Siljestrom, M. Rapid enzymatic assay of insoluble and soluble dietary fibre. Journal of Agricultural and Food Chemistry, 3, 476–482, (1983).

[35] L. Prosky, L., Asp, T.F. Schweizer, J.W. DeVries, I..Furda, Determination of insoluble, soluble, and total dietary fiber in foods and food products: interlaboratory study. J. Assoc. Off. Anal. Chem. 71 (5), 1017–1023, 1988.

[36] V. L. Singleton, R., Orthofer, R. M..Lamuela-Raventos, Analysis of total phenols and other oxidation substrates and antioxidants by means of Folin–Ciocalteu reagent. Methods in Enzymology, 299, 152–178, (1999).

[37] A..Escarpa, M.C. Gonzalez, Approach to the content of total extractable phenol compounds from different food samples by comparison of Chromatographic and spectrophotometer methods. Anal. Chim. Acta., 427,119–127, (2001).

[38] A. Arvouet-Grand, B. Vennat, A. Pourrat, P. Legret, Standardisation d'un extrait de propolis et identification des principaux constituants. Journal de Pharmacie de Belgique, 49 (6), 462-468, (1994).

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

[40] R..Re, N.. Pellegernie, A. Proteggente, Pannala, A., Yang, M., & C. Rice-Evans, Antioxidant activity appliying and improved ABTS radical cation decolorization assay, Free Radical Bio. Med., 26,1231-1237, (1999).

[41] F. Stintzing, K..Herbach, M.,.Mosshammer, R..Carle, W. Yi, S.,Sellappan, C. Akoh, R.. Bunch, R.. Felkers, x Color,betalain pattern, and antioxidant properties of cactus pear (Opuntia spp.) clones. J. Agric. Food Chem. 53 (2), 442–451, 2005.

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

[43] M..El Gersifi, les défauts des produits de patisserie et biscuie au cours du stockage: la prevention par la formulation. Industrie Alimentaires et Agricole, 82–88, 1998.

[44] M.E.. Malainine, A. Dufresne, D. Dupeyre, M.R..Vignon, M..Mahrouz, First evidence of weddelite crystallites in Opuntia ficus-indica parenchyma. Z. Naturforsch./Biosci. 58, 812–815, 2003.

[45] H.K. .Gulia, B.C. Sharma, A. Sarkara, A. Upadhyay,, Shitandi, Changes in physico-chemical and functional properties during convective drying of aloe vera (Aloe barbadensis) leaves, food and bioproducts p rocessing 8 8 161–164, (2 0 1 0)

[46] M.A. Ayadi , W. Abdelmaksoud, M. Ennouri, H. Attia, Cladodes from Opuntia ficus indica as a source of dietary fiber: Effect on dough characteristics and cake making, Industrial Crops and Products 30 40–47, (2009)

[47] C. Sàenz, Cladodes: a source of dietary fiber. J. Prof. Assoc. Cactus Dev., 117–123, 1997.

[48] F. Shahidi, P.K. Janitha, P.D. Wanasundara, Phenolic antioxidant, critical previous term reviews next term. Food Sci. Nutr. 32, 67–103, 1992.

[49] R.S. Harris, E. Karmas, Nutritional Evaluation of Food Processing. The Avi Publishing Company Inc., Westport, CT, 1977.

[50] R..Chavez-Santoscoy, J. Gutierrez-Uribe, S. Serna-Saldı´var, Phenolic composition, antioxidant capacity and in vitro cancer cell cytotoxicity of nine prickly pear (Opuntia spp.) juices. Plant Foods Hum. Nutr. (Formerly Qualitas Plantarum) 64 (2), 146–152, 2009.

[51] F. Burret, P. Lebreton, B. VoirinLes aglycones flavoniques des Cactees: distribution, signification. Journal Natural Products, 45: 687–693, (1982)

[52] Weirong Cai , Xiaohong Gu and Jian Tan, Extraction, Purification, and Characterisation of the Flavonoids from Opuntia milpa alta Skin, Czech J. Food Sci , Vol. 28, No. 2: 108–116, 2010.

[53] K. E. Heim, A. R. Tagliaferro, & D. J. Bobilya, Flavonoid antioxidants: chemistry, metabolism and structure-activity relationships. The Journal of Nutritional Biochemistry, 13(10), 572–584, (2002).

[54] C. A. Rice-Evans, N. J. Miller, & G. Paganga,. Structure-antioxidant active relationship of flavonoids and phenolic acids. Free Radical Biology and Medicine, 20(7), 933–956.Paganga, (1996)

[55] G.. Ginestra, M. L. Parker, R. N. Bennett, J. Robertson, G. . Mandalari, A. Narbad, Anatomical, chemical and biochemical characterization of cladodes fromprickly pear (Opuntia ficus-indica (L.) Mill). Journal of Agricultural and FoodChemistry, 57, 10323–10330, .(2009).

[56] T. Guevara-Figueroa, H. Jimenez-Islas, M. L. Reyes-Escogido, A. G. Mortensen, B. B. Laursen, , L. Linet al. x Proximate composition, phenolic acids, and flavonoids characterization of commercial and wild nopal (Opuntia spp.). Journal of Food Composition and Analysis, 23, 525–532, 2005

[57] L. Santos-Zea, , J. A Gutierrez-Uribe.& S. O Serna-Saldivar,. (Comparative analyses of total phenols, antioxidant activity, and flavonol glycoside profile of cladode flours from different varieties of Opuntia spp. Journal of Agricultural and Food Chemistry, 59, 7054–7061, 2011).

[58] S. F. Price, P. J. Breen, M.,. Valladao, & B. T. Watson, Cluster sun exposure and quercetin in Pinot Noir grapes and wine. American Journal of Enology and Viticulture,46, 187–194, (1995).

[59] A. J. Stewart, Bozonnet, S., Mullen, W. Jenkins, G. I., Lean, E., & , A. Grozier (2000). Occurrence of flavonols in tomatoes and tomato-based products. Journal of Agricultural and Food Chemistry, 48, 2663–2669.

[60] G..Schirrmacher, W. H.,.Schnitzler, & J. Graßmann, Determination of secondary plant metabolites and antioxidative capacity as new parameter for quality evaluation —Indicated by the new Asia salad Gynura bicolor. Journal of Applied Botany and Food Quality, 78, 133–134, (2004).