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Chemical Composition and Antioxidant Activity of seeds oils and fruit juice of Opuntia Ficus Indica and Opuntia Dillenii from Morocco

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Abstract

This study provides basic information on the mineral composition of the seeds and antioxidant activity in seeds oils and fruit juices of cactus belonging to two species *Opuntia ficus indica* and *Opuntia dillenii*, from Morocco (Oujda), in order to evaluate the nutritional value of the *Opuntia* extracts. Minerals determined from dry seeds of *Opuntia ficus indica* and *Opuntia dillenii* were: calcium 480.93 and 408.28; phosphorus 1417.59 and 970.15; potassium 304.51 and 201.96; magnesium: 316.59 and 240.30; sodium: 48.33 and 18.18; zinc: 70.77 and 78.26 mg/100g respectively. The main fatty acids of *Opuntia ficus indica* and *Opuntia dillenii* seed oil were respectively: linoleic acid: 58.79 and 79.83%, Palmitic acid: 11.18 and 13.52%. The antioxidant activity of *Opuntia ficus indica* and *Opuntia dillenii* seed oils and fruit juices were assessed by means of 2, 2-diphenyl-1-picrylhydrazyl (DPPH) radical-scavenging assay and ascorbic acid test. The results showed that the antioxidant activities of *Opuntia ficus indica* and *Opuntia dillenii* seed oil (IC50 = 19.79 ± 0.023 and 27.21 ± 0.075 µL/mL) are higher than that of the reference ascorbic acid (IC50 = 16.56 ± 0.019 µg/mL). However, the *Opuntia dillenii* juice presents antioxidant activity more important than this of *Opuntia* seed oil and ascorbic acid. It possessed strong antioxidant activity (IC50 = 8.18 µL/mL). The antioxidant activity of the seed oil and juice were also found to be concentration-dependent.

Keywords: Opuntia Ficus Indica, *Opuntia Dillenii*, minerals, juice, seed oil, antioxidant activity.

1. Introduction

There has been a recent trend in consumer demand for foods with higher nutritional value, as well as with health benefits, which has spawned a new category of ‘functional foods’. The health benefits include disease prevention [1]. Functional compounds are those that help to prevent or treat disease and/or improve physical or mental performance [2]. The fruit of cactus pear provide interesting sources of functional compounds, including total phenols, flavonoids, carotenoids, dietary fibers, betalains, taurine and linoleic acid), vitamins (C, E, group-B and β-carotene), minerals (potassium, calcium, phosphorus and selenium), and free amino acids (proline, phenylalanine, alanine, lysine and histidine. These compounds are valued for their contribution to a healthy diet and also as ingredients for designing new foods.

Minerals are inorganic nutrients, usually required in small amounts from less than 1 to 2500 mg per day, depending on the mineral. As with vitamins and other essential food nutrients, mineral requirements vary with animal species. For example, humans and other vertebrates need large amounts of calcium for construction and maintenance of bone and normal function of nerves and muscles. Phosphorus is an important constituent of adenosine triphosphate (ATP) and nucleic acid and is also essential for acid-base balance, bone and tooth formation. Red blood cells can't function properly without iron in hemoglobin, the oxygen-carrying pigment of red blood cells. Iron is also an important component of the cytochromes that function in cellular respiration. Magnesium, copper, selenium, zinc, iron, manganese and molybdenum are important co-factors found in the structure of certain enzymes and are indispensable in numerous biochemical pathways. Vertebrates need iodine to make thyroid hormones. Sodium and potassium are important in the maintenance of osmotic balance between cells and the interstitial fluid. Magnesium is an important component of chlorophyll in plants [3]. Being high in nutritional and bioactive phytochemicals, cactus pear fruit can be used both as a potential source of natural antioxidants and as a direct functional food [4].

The fruits of *opuntia ficus-indica* and *opuntia dillenii*, have anti-inflammatory and analgesic effects [5], anti-hyperglycemia and hypocholesterolemic effects [6-7]. Butera et al. [8] reported that prickly pear (*opuntia ficus-indica*) white fruit extracts showed the highest protective effects of all models of lipids oxidation due to its high content of betalains, which contributes to the antioxidant activity of prickly pear fruit. Kanner et al. [9] also specified betalain as a new class of dietary cationized antioxidant.

Recently, there has been an increased interest in the antioxidant activity and health-improving capacity of cactus pear, and the antioxidant capacity of the pulp of cactus-pear fruits has been assessed [10-14]. Since, some by-product constituents may be extracted and used as additives in food preparations or in the pharmaceutical and cosmetic sectors, the use of processed fruit by-products for human consumption has increased significantly in recent years. Cerezal and Duarte [15] used the cactus pear pericarp to formulate marmalade. The seed meal seems to have potential use as a dietary fiber source for human consumption, for the extraction of oil [16-18]. However, the seeds of *opuntia dillenii* and *opuntia ficus indica* possesse the potential as a high-quality edible oil of benefit to health containing a high amount of unsaturated fatty acids, as well as providing valuable natural antioxidants for the pharmaceutical industry. To the best of our knowledge, there is limited literature on the study of the chemical composition of seed oil and fruit juice from *opuntia dillenii* and there antioxidant activities.

The main objectives of this study were: 1) determine the mineral composition of the seeds from *opuntia ficus indica* and *opuntia dillenii*; 2) evaluate the free radical DPPH scavenging capacities of the seed oils and fruit juices for the two species. To the best of our knowledge, studies on the chemical composition of seed oil from *opuntia dillenii* and its antioxidant activity have rarely been reported [19]. The mineral composition of the seed was analyzed by atomic emission spectrometry ICP AES. Furthermore, antioxidant activity of samples was determined by means of 2,2- diphenyl-1-picrylhydrazyl (DPPH) radical-scavenging assay and ascorbic acid bleaching test.

2. Material and Methods

2.1. Chemicals and Reagents

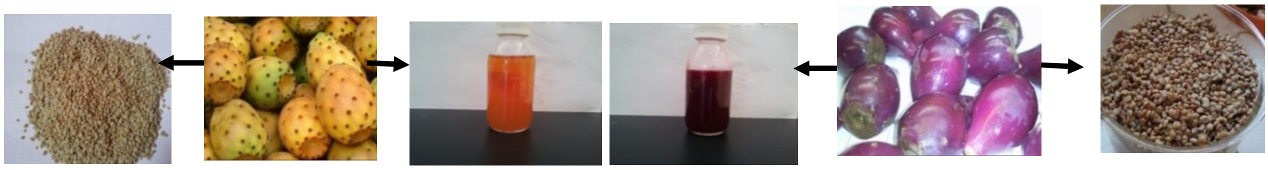
Hexane was purchased from (E. Merck). 2,2-diphenyl-1-picrylhydrazyl (DPPH) and ascorbic acid were purchased from (Sigma-Aldrich GmbH, Germany). All other chemicals and solvents were of the highest analytical grade and used as supplied.

2.2. Sample collection and preparation

The mature fruits of prickly pear, *Opuntia Ficus Indica* and *Opuntia Dillenii* were collected respectively, in June and February 2012 from the same area (Oujda, Morocco). They were washed and peeled. The pulp was dotted with several small seeds (Figure 1). The pulp was mixed for five minutes in a Moulinex blender in order to separate seeds from the juice after passing through a sieve. Seeds were washed thoroughly with water, dried at room temperature, ground to a fine powder using a Moulinex coffee grinder and stored at -20 °C as well as the fruit juice.

2.3. Extraction of seed oil

Ground seed (10 g) was used for lipid extraction in a 250 mL round-bottom flask. Organic solvent (25 mL) was added and the mixture was stirred under ambient temperature for 2 h. After filtration, the solvent was concentrated on a rotary evaporator under reduced pressure at 40°C. Oil was dried with Magnesium sulfate and left overnight in a refrigerator at 4°C.



OFI seeds OFI fruit OFI juice OD juice OD fruit OD seeds

Figure 1: *Opuntia Ficus Indica* and *Opuntia Dillenii* fruits, seeds and juices

2.4. Mineral composition analysis

150 mg of *opuntia ficus indica* and *opuntia dillenii* seeds were dried and ground. Mineralization was carried out by using 2 ml of nitric acid (70%), 3 ml of hydrofluoric acid and 2 ml of chlorydric acid. The mixture was then boiled at about 100-110 °C for 15 hours. After cooling, 25ml of 2M HCl solution were added to the mixture. Minerals contents were determined using an inductively coupled plasma atomic emission spectrometer (Ultima 2 - JobinYvon).

2.5. Free Radical Scavenging (FRS) Activity

2.5.1. Determination of antioxidant activity

The seed oils and fruit juice obtained were subjected to screening for their possible antioxidant activities. The antioxidant activity was assessed using 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical-scavenging assay and ascorbic acid bleaching test. All the data were the averages of triplicate determinations of three independent tests.

2.5.2. Free Radical Scavenging (FRS) Activity experimental

The free radical scavenging activity of *opuntia ficus indica* and *opuntia dillenii* seed oil and fruit juice was measured according to the method of Brand-Williams et al. [20] with some modifications using the stable 2, 2-diphenyl-1- picrylhydrazyl radical (DPPH). Briefly, 0.6 mL of various concentrations of the each extracts were and mixed with 2.4 mL of a 0.004% methanol solution of DPPH (Sigma-Aldrich). After a 30 min incubation period at room temperature, the absorbance was read against a blank at 517 nm. Inhibition of free radical DPPH in percent (I%) was calculated in following way:

$$I\% = [(A_{blank} – A_{sample}) /A_{blank}] \times 100$$

Where A_{blank} is the absorbance of the control reaction (containing all reagents except the test compound), and A_{sample} is the absorbance of the test compound.

The DPPH radical-scavenging activity was estimated from the difference in absorbance, with or without tested compounds or extracts, and expressed as a percentage of DPPH scavenged in solution. The IC50 value represents the concentration of an individual compound required to quench 50% of DPPH under experimental conditions. All the tests were done in triplicate.

3. Results and discussion

3.1. Chemical composition of Fatty Acids seed oils

Two species of prickly pear from Morocco, *Opuntia ficus indica* and *Opuntia dillenii* were investigated in a previous work by Ramdani et al. [21] for fatty acids, sterols and vitamin E composition. Oils are obtained by hexane maceration under ambient temperature and analyzed by GC-MS. The main fatty acids of *Opuntia ficus indica* and *Opuntia dillenii* seed oil were respectively: linoleic acid: 58.79 and 79.83%, Palmitic acid: 11.18 and 13.52%. In both oils, stearic acid was present at low percentage: 1.50 and 2.75%. The content of unsaturated fatty acids was high, at 58.79% and 79.83% for *Opuntia ficus indica* and *Opuntia dillenii*, respectively. The sterolic fraction was composed by β-sitosterol: 21.93 and 2.80%, campesterol: 3.75 and 0.51%, stigmasterol: 1.64 and 0% and fucosterol: 0 and 0.27% respectively. The sterol marker, β-sitosterol, accounted for 80.27% and 78.21% of the total sterol content in *Opuntia ficus indica* and *Opuntia dillenii* seed oils. In both oils, vitamin E, γ-tocopherol was present with low quantities 1.23% and 0.29% of total lipids respectively.

3.2. Mineral composition of Opuntia ficus indica and Opuntia dillenii dry seeds

The minerals contents of cactus seeds cultivated in the area of Oujda in the east of Morocco were summarized in Table 1.

Table 1: Mineral composition of *Opuntia ficus indica* and *Opuntia dillenii* dry seeds.

	minerals	**This study (mg /100g dry seeds)**		**Literature (mg/100 g, dry seeds)**	
		O.Ficus.I	***O.Dillenii***	***O.Ficus.I*** [22]	***O.Ficus.I*** [23]
Macro elements	Ca	**480.93**	**408.28**	21,20	16.20
	Mg	**316.59**	**240.30**	9.85	74.80
	Na	48.33	18.18	0.54	67.60
	K	**304.51**	**201.96**	78.60	163
	P	**1417.59**	**970.15**	-	152
Trace elements	Fe	2.76	1.98	-	9.45
	Cu	1.03	1.18	-	0.32
	Zn	**70.77**	**78.26**	-	1.45
	Mn	5.18	4.35	-	trace
	Cr	1.38	1.58	-	-
	Ni	2.07	2.76	-	-
	Mb	-	-	-	-
	Si	-	-	-	-

Opuntia seeds were considered a good source of minerals, our results showed that the macroelements contents of *Opuntia ficus indica* and *Opuntia dillenii* seeds were higher than those reported in the literature by Nebbache et al. [22] and El-Mostafa et al. [23] Phosphorus was the major element at 1417.59 and 970,15 mg/100 g dry seeds followed by calcium at 480.93 and 408.28; magnesium at 316.59 and 240.30; potassium at 304.51 and 201.96 and sodium at a lower content 48.33 and 18.18 mg/100 g of *Opuntia ficus indica* and *Opuntia dillenii* seeds respectively (Figure 2). Sodium is beneficial for people with kidney problems and hypertension [24, 25].

Among oligoelements, zinc contents at 70.77 and 78.26 mg/100 g of *Opuntia ficus indica and Opuntia dillenii* seeds respectively were found to be higher than the other minerals like iron, manganese, nickel, chrome and copper content (Figure 3). The difference in the minerals amounts reported by authors in different countries could be attributed to: the location of plants; the agronomy of cultivation; the application of fertilizers and irrigation use; climate; and genetic differences between the varieties [26]. Zinc is distributed widely in plant and animal tissues and occurs in all living cells. It functions as a cofactor and is a constituent of many enzymes like lactate dehydrogenase, alcohol dehydrogenase, glutamic dehydrogenase, alkaline phosphatase, carbonic anhydrase, carboxypeptidase, superoxide dismutase, retinene reductase, DNA and RNA polymerase. Zn dependent enzymes are involved in macronutrient metabolism and cell replication [27, 28].

3.3. DPPH Radical Scavenging and Antioxidant Activities of seeds oils and fruit juices

Results of free radical scavenging activity of cactus seeds oils and fruit juices of *Opuntia ficus indica* and *Opuntia dillenii* are given in Table (2). Data in the table indicated that the DPPH scavenging activities (%) were increased significantly with increasing the concentration of the cactus seed oils and fruit juices from 5 to 20µL/mL.

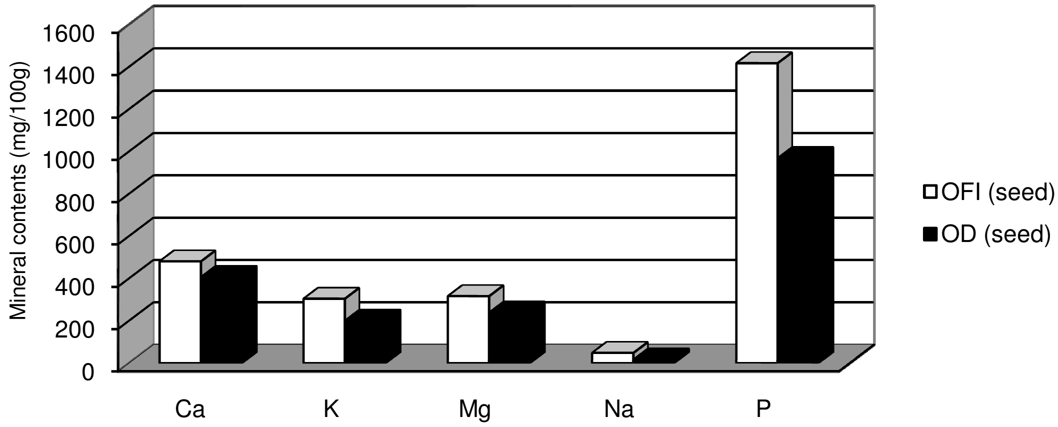


Figure 2: Macro-elements content of *Opuntia ficus indica* and *Opuntia dillenii* seeds

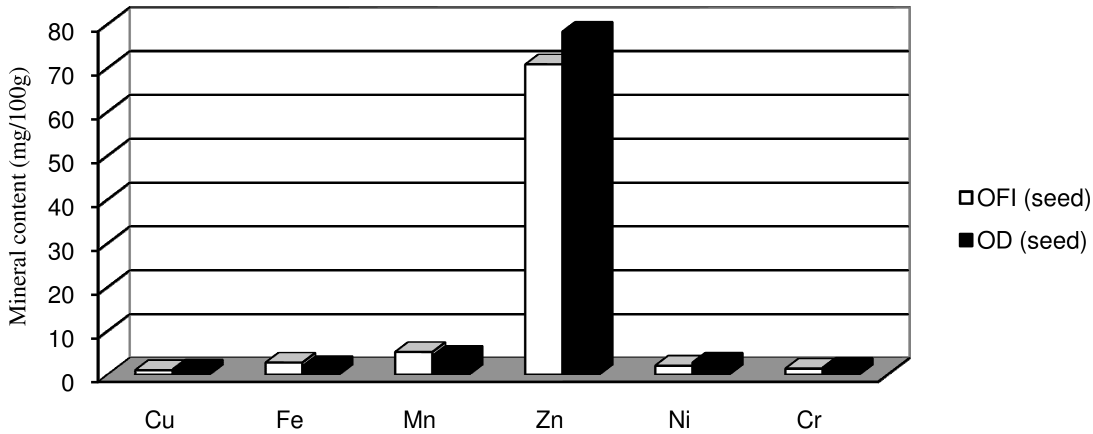


Figure 3: Oligo-elements content of *Opuntia ficus indica* and *Opuntia dillenii* seeds

Table 2: Antioxidant activity of seeds oils and fruit juices extracts of *Opuntia ficus indica* and *Opuntia dillenii* determined by DPPH˙ radical scavenging assay

Inhibition ratio (%)					
Concentration (µL/ml)	**Oils**		**Juices**		**Ascorbic acid(a)**
	O.Ficus.I	***O.Dillenii***	***O.Dillenii***	***O.Ficus.I***	
5	24.84 ± 0.070	21.04 ± 0.071	39.15 ± 0.095	32.17 ±0.080	21.02 ± 0.066
10	31.04 ± 0.086	26.19 ± 0.076	56.11 ± 0.080	42.15 ± 0.046	29.96 ± 0.091
15	37.62 ± 0.070	29.81 ± 0.066	73.54 ± 0.164	50.56 ± 0.083	40.15 ± 0.060
20	53.15 ± 0.085	42.60 ± 0.061	91.94 ± 0.031	68.51 ± 0.051	63.85 ± 0.064
equations	1.830x +13.783	1.366x + 12.838	3.516x + 21.238	2.349x + 18.992	2.774x + 4.075
coefficient R^2	0.942	0.919	0.999	0.968	0.939
IC50 (n=3)	**19.79 ± 0.023**	**27.21 ± 0.075**	**8.18 ± 0.010**	**13.20 ± 0.013**	**16.56 ± 0.019**

(a) Concentration of ascorbic acid given in µg/mL

The results from table 2 showed notable antioxidant activity of *Opuntia ficus indica* and *Opuntia dillenii* seed oils (Figure 4). IC50 values (concentration of sample required for 50% inhibition of DPPH radical scavenging activity, figure 5) for the inhibition of DPPH were 19.79 and 27.21 µL/mL, respectively, which were higher than that of the reference ascorbic acid (IC50 = 16.56 µg/mL).

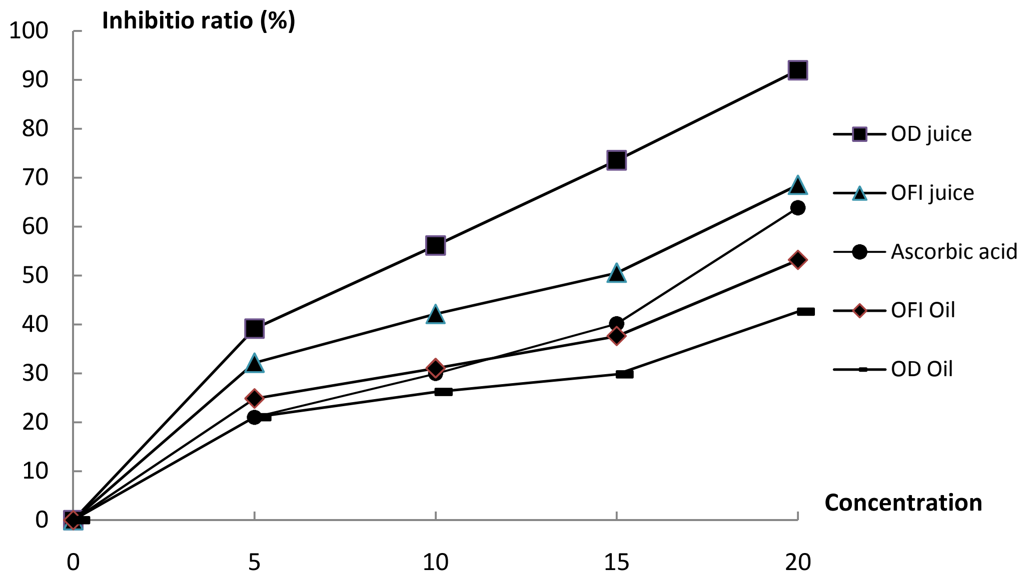


Figure 4: Antioxidant activity of cactus seeds oils, fruits juices extracts and ascorbic acid determined by DPPH˙ radical scavenging assay. Concentration: *Opuntia ficus indica*, *Opuntia dillenii* oils and juices (µL/mL); Ascorbic acid (µg/mL)

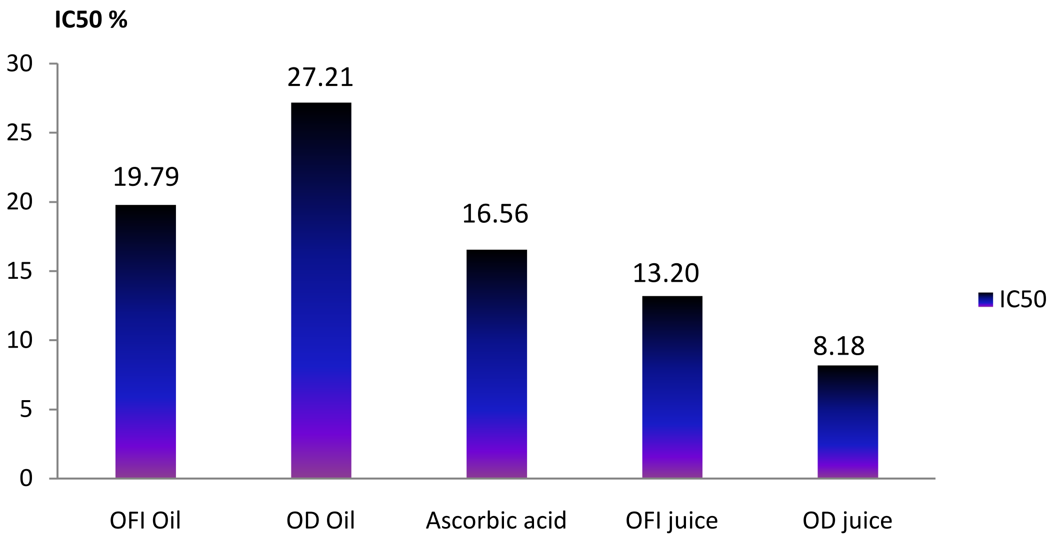


Figure 5: IC50 of *Opuntia ficus indica* and *Opuntia dillenii* oils and *Opuntia ficus indica* and *Opuntia dillenii* juices and ascorbic acid

However, Yuan-Gang [29] reported that seed oil from *O. dillenii* exhibited notable DPPH radical-scavenging activity, with an efficacy slightly lower (IC50 = 11.43%) than that of the reference ascorbic acid (95.21 at 4 mg/mL). Interestingly, we observed that the *O. dillenii* and *O. ficus indica* fruits juices presented a strong antioxidant activity (IC50 = 8,18 and 13,20 µl/mL respectively), about three times higher than this of *O. dillenii* oil and two times higher than this of ascorbic acid. Otherwise, Abd El-Razek et al. [30] reported that the DPPH scavenging activity of 50 (µl/ml) of *O. ficus indica* fruit Juice presented a low value 19.34. In contrast, in our investigation, *O. ficus indica* fruit Juice was higly active, the antioxidant activity attained 32.17 % for only 5 µl/mL.

Chang et al. [31] reported that methanolic extracts of *O. dillenii* fruit possessed notable antioxidant activity, and the activities of seed extracts were stronger than those of peel and pulp extracts. Their results also demonstrated that the higher amounts of polyphenols and flavonoids in the seeds of *O. dillenii* may contribute to the stronger antioxidant activity of the seeds. However, the seeds of *O. dillenii* are rich in oil, and the high amount of unsaturated fatty acids may also possess potentially notable antioxidant activity. Moreover, Maataoui et al. [32] showed that pigments have 1.5 times higher antioxidant activity than ascorbic acid. Butera *et al.* [8] reported that prickly pear (*O. ficus-indica*) white fruit extracts showed the highest protective effects of all models of lipids oxidation due to its high content of betalains, which contributes to the antioxidant activity of prickly pear fruit.

Consumption of foods rich in natural antioxidants has been reported as being protective against certain types of cancer and may also reduce the risk of cardiovascular and cerebrovascular events [33]. Currently, little information is available on the chemical composition and antioxidant activity of seed oil from *O. dillenii* in the literature.

Conclusion

This study showed that *Opuntia Ficus Indica* and *Opuntia Dillenii* seeds were rich in macroelements. Cactus fruit was considered as a good source of minerals.

The *O. ficus indica* and *O. dillenii* seeds oils possessed the potential as a high-quality edible oil of benefit to health. *O. Dillenii* seeds oil was found to be more unsaturated: 79.83% than *O. Ficus Indica*: 58.79%. Linoleic acid was the dominating fatty acid with an exceptional level, up to 79.83% for *O. dillenii* and 58.79% for *O. ficus indica* oil.

Results of this study showed high antioxidant activities of *O. ficus indica* and *O. dillenii* seeds oils. However, the *O. dillenii* and *O. ficus indica* fruits juices presented strong antioxidant activities more important than Vitamin C. We conclude that the value of *Opuntia* cactus pear by-products as a good and cheap source of minerals, unsaturated fatty acids and natural antioxidants could be industrially exploited. However, before considering incorporation of cactus pear by-products as a dietary complement or as natural food antioxidant, it is necessary to carry out further studies in order to test their in vivo activity, bioavailability, and toxicity also.

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