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Effect of phenolic compounds and betalain pigments on the antioxidant capacity of Moroccan prickly pear juices

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Abstract

Juices of nine prickly pears cultivars (*Opuntia ficus-indica L.*) were characterized in terms of phenolics and betalains pigment content. The antioxidant activity of juice was tested by means of two different methods: the 2,2-diphenyl-1 picrylhydrazyl (DPPH) methods, and the 2,2-azinobis(3-ethyl-benzothiazoline-6-sulfonic acid) (ABTS) methods. Juices contained total phenolics ranging from 354.37 to 643.66 µg gallic acid eq/g, betaxanthins (15.84 to 51.33 mg indicaxanthin/l), betacyanins (52.04 mg betanin/l for red juice) and antioxidant capacity (DPPH) ranging from 52.48 to 135.96µg/ml respectively. The total phenolic contents were highly correlated with ABTS (R^2 = 0.868) and DPPH (R^2 = 0.959) values. The phenolics compounds contribute more significantly to the total antioxidant capacity than betalain pigments. Therefore, the total phenolic contents can serve as a useful indicator for the antioxidant activities of prickly pear juices.

Keywords: Prickly pear juices, phenolics, betaxanthins, betacyanins, antioxidant activity.

1. Introduction

Cactus pear "*Opuntia ficus indica* L.", a member of the Cactaceae family, is widely distributed in Morocco and grows in many other parts of the world, such as American hemispheres, Africa, Australia and the Mediterranean basin and cultivated in dry regions as an important nutrient and food source [Lamghari, R; El Kossori, *et al.* 1998, Trombetta, D *et al.* 2006, Ennouri, M *et al.* 2005].
In some countries, cactus-pear juice is consumed at home, in vegetarian restaurants, or in local health-food stores.
The nutritional importance of cactus pear fruit juice is mainly due to the content of sugars (12–15%), ascorbic acid, fibres and free amino acids (particularly proline, glutamine and taurine) [Stintzing, F.C *et al.* 2001]. Other components are present such as lipids (0.1%), phenolics (0.05%) [Alfredo Cassano, *et al.* 2010], proteins (0.6%), organic acids and minerals include calcium, potassium, and magnesium (490, 2200, and 850 ppm, respectively) [Hernandez-Perez, T *et al.* 2005].
The fruit juice is also characterised by a high content of betalain, a widely used natural colorant in the food industry. The two main betalain pigments are the purple-red, betacyanins and the unique yellow indicaxanthin [El Gharras *et al.* 2008; Alfredo Cassano *et al.* 2010].

Betalain pigment has recently been shown as an antioxidant in a number of model systems of lipid oxidation [Kanner, J *et al.* 2001].

Antioxidants are compounds that can delay or prevent the oxidation of lipids or other molecules by inhibiting the initiation or propagation of oxidative chain reactions [Velioglu, Y *et al.* 1998]. Plants are potential sources of natural antioxidants, and certain species are particularly significant because they may be used for the production of raw materials or preparations containing photochemical with significant antioxidant capacities and health benefits [Exarchou, V *et al.* 2002].

The anti-oxidative effect is mainly due to phenolic compounds, such as flavonoids, phenolic acids, tannins and phenolic diterpenes [Shahidi, F *et al.* 1992, Chung, K. T *et al.* 1998, Pietta, P. G *et al.* 2000]. They interfere with the oxidation process by reacting with free radicals, chelating catalytic metals, and scavenging oxygen. The effect of juices on antioxidant activity could be a result of the types of polyphenolics they contained [Cai *et al.* 2003]. Many natural flavonoids have considerably higher antioxidant potentials than nutrient antioxidants, such as vitamin C (ascorbic acid) and vitamin E and dietary

antioxidants, such as carotenoids [Vinson, *et al.* 1995]. Several methods are available to evaluate antioxidant activities of natural compounds in foods or biological systems. Two methods commonly used in antioxidant activity assays are the DPPH and ABTS procedures, which use 2,2- diphenyl-1-picrylhydrazyl (DPPH) and 2,2-azinobis(3- ethyl-benzothiazoline-6-sulfonic acid) (ABTS) as free radical generators, respectively.

The aim of the present study was to characterize the antioxidant activity and capacity of nine different cultivars of prickly pears juices harvested from different geographic regions in Morocco, and to study the correlation between the antioxidant component and the antioxidant capacity of Moroccan prickly pear juices.

2. Material and methods

Nine cultivars of prickly pears fruits, grown in different area in Morocco, were selected at maximum full maturity without being overripe: yellow species from *Doukkala; Tamellalet; Ras Elain; Ben Guerir; Ait Baamrane; Skhour Rehamna; Alkalaa* and both species red and yellow from *Khouribga* (Figure 1 and Table 1).

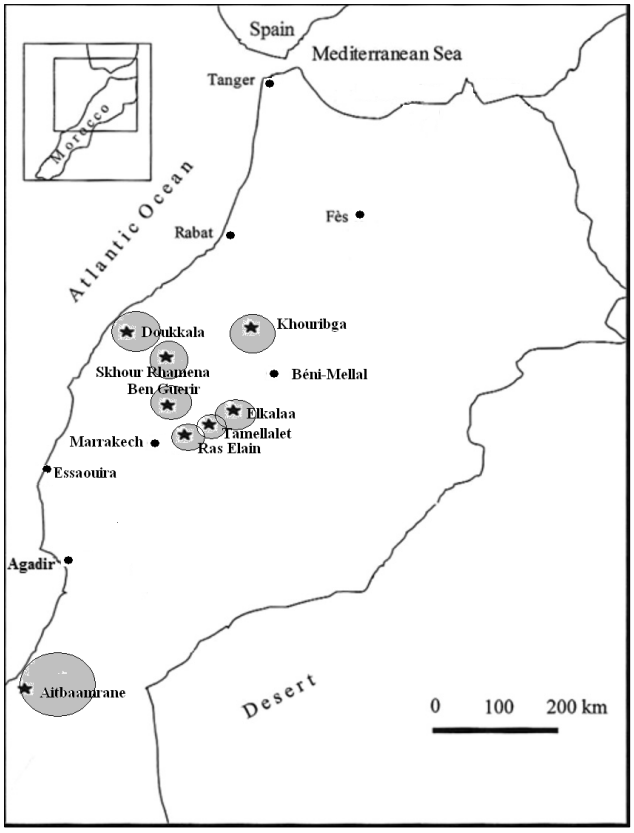

Figure 1. Repartition map of Moroccan prickly pears cultivars used in the study

Table 1. Geographic of provenance of prickly pears fruits used in the study

Area	*Latitude N*	*Longitude W*
Khouribga	32°53’	6°54’
Skhour Rehamna	32°29’	7°55’
Alkalaa	32°02’	7°24’
Tamellalet	31°49’	7°30’
Ras Elain	31°48’	7°34’
Ben Guerir	32°14’	7°57’
Doukkala	32°35’	8°39’
Ait Baamrane	29°23’	10°10’

For each species, three different lots of fruits were harvested at the same season, carefully washed with water to remove the glochids and the obtained juice was centrifuged (4000 rpm, 30min at 4°C) and the supernatant juice was stored at -20°C before being used. For analysis, triplicate determinations were performed on each sample; data shown later represent the means of three measurements.

2.1. Determination of total phenol content:

The total phenolic contents of prickly pear juice samples were determined using a modified Folin-Ciocalteu method cited by [Wolfe *et al.* 2003]. A 50µl aliquot of a known dilution of the extract was added to the test tube and combined with 500 µl of Folin–Ciocalteus reagent. The tubes were vortexed for 15 seconds. About 1.5 ml of 7% sodium carbonate solution was then added to the test tubes, and the mixture was diluted to 5 ml with distilled and de-ionized water. The tubes were stored at dark and colour was developed for 90 min and the absorbance was measured at 727 nm using the (UV-Vis 1650PC Shimadzu, JAPON). The measurement was compared to a standard curve of prepared gallic acid solutions and expressed as gallic acid equivalents in micrograms per gram of juice. Triplicate determinations were performed on each sample; data shown later represent the means of three measurements.

2.2. Determination of Betalains contents:

To extract pigments, the juice was homogenized in methanol (analytical grad, Aldrich, Deisenhofen, Germany) (1:5 w⁄v) and magnetically stirred for, 1 min. The homogenate was filtered through a 0.45µl mesh nylon filter (Whatman). UV-Vis absorbance spectra of the extracts were recorded using a spectrophotometer (Shimadzu UV Visible 1650PC Shimadzu, JAPON)) equipped with one-cm optical-path quartz cells.

Quantification of betalains was carried out in triplicate applying the molar extinction coefficients of betanin (ε=60,000 L/mol cm in H_2O; λ=532 nm; MW=550 g/mol)

and indicaxanthin (ε=48,000 L/mol cm in H_2O; λ=482 nm; MW=308 g/mol). The betalain content (BC), expressed as mg/L, was calculated by using the following equation:

$$BC = A \times DF \times MW \times 1000/ \varepsilon \times L$$

Where A is the absorption at 532 and 482 nm for betacyanins and betaxanthins, respectively (figure 2 and figure 3); DF is the dilution factor and L the path-length of the 1-cm cuvette. For MW and ε, the molecular weights and extinction coefficients of the representative compounds betanin and indicaxanthin have to be considered, respectively [Cai, Y. Z *et al.* 1999; Alfredo Cassano *et al.* 2010].

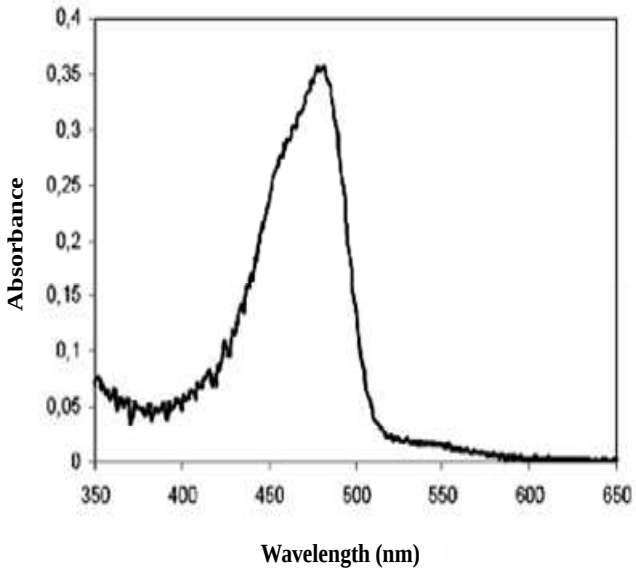

Figure 2: UV-Visible absorbance spectrum of the methanolic extract of the yellow cactus pear juice

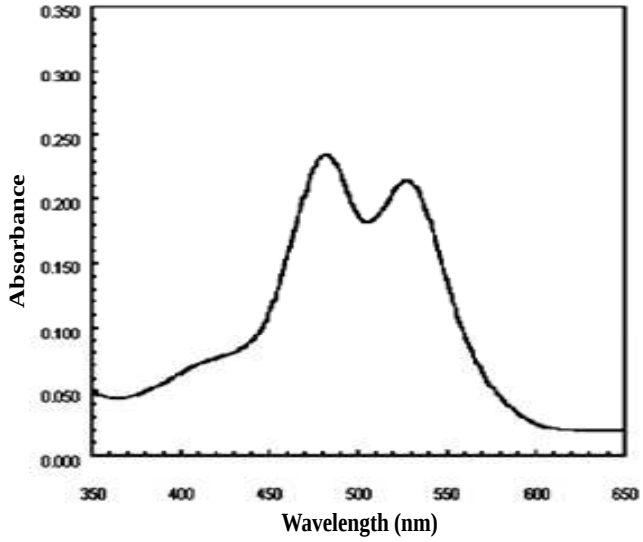

Fig. 3: UV-Visible absorbance spectrum of the methanolic extract of the reddish cactus pear juice.

2.3. Determination of antioxidant activity

- *Assay of DPPH radical scavenging activity:*

The free radical-scavenger activity was determined by the DPPH assay, as described previously by Campos *et al.* [Campos, M. G *et al.* 2003]. The antiradical activity of extracts was evaluated using a dilution series, in order to obtain a large spectrum of sample concentrations. This involved the mixing of 250µl of DPPH• solution (6 mg of DPPH in methanol) with an appropriate amount of extract or compound, followed by homogenization. After incubation in the dark at room temperature for 30min, quantification of the remaining DPPH• radicals was recorded by using absorption set at 517 nm. Three analytical replicates (n=3) were carried out on each extract for antiradical activity determinations. Measurements were averaged, and results are given as mean ± standard deviation (S.D.). Antiradical efficiency was established using regression analysis at a 95% significance level ($P<0.05$). Results are presented in IC50 values, which represent the weight of sample required to scavenge 50% of the DPPH radicals available.

- *Assay of ABTS radical scavenging activity:*

The ABTS assay was carried out as described by Re *et al.* [Pellegrini, N *et al.* 1999]. For ABTS•+ generation from ABTS salt, 2.5 mM of potassium persulfate ($K_2S_2O_8$) was reacted with 7 mM ABTS salt, for 16 h at room temperature in the dark. The resultant ABTS•+ radical cation was diluted with methanol, to give an absorbance of around 0.70 ± 0.02 at 730 nm. The standard and sample prickly pear juice were diluted with the ABTS.+ solution to a total volume of 3 ml and allowed at darkness for 6 min. Absorbance was measured at 730 nm and the free-radical-scavenging activity was expressed as mmol of Trolox Equivalent per gram of Dry Weight (DW) of sample (mmol TE/g DW).

3. Results and discussion

The total phenolics contents of the juice of the nine cultivars of prickly pears varied from 354.37 to 643.66 µg GAE/g of juice (Table 2). The cultivar from *Ait Baamran* contained the highest amount of total phenols (643.66 µg GAE/g of juice) followed by *Alkalaa* cv. (632.11 µg GAE/g of juice). The juice from *Khouribga cv.* contained the lowest amount. The other cultivars from *Skhour Rhamna*, *Ras Elain*, *Tamellalet* and *Ben Guerir* contained comparable amounts. The juices of Moroccan origin contained higher phenolic amount than the juices from Mexican prickly pears ranging from 55.4 to 226.3µg GAE/g of juice [R. A. Chavez-Santoscoy *et al.* 2009].

Table 2:

Total phenols, betaxanthins, betacyanins pigments and antioxidant activity of juices extracted from nine Moroccan prickly pears

Cultivars	Total phenols µg GA eq./g of juice	Betaxanthins mg indicaxantin/l of juice	Betacyanins mg betanin/l of juice	ABTS mM TE/g of Dry Weight of juice	DPPH EC50 µg/ml of juice
Skhour Rhamna	476.37±5.13	37.66±4.77	-	0.17±0.02	91.2±3.63
Alkalaa	632.11±5.50	42.83±3.00	-	0.24±0.02	65.86±5.20
Yellow Khouribga	354.37±5.37	26.68±2.12	-	0.16±0.03	135.96±12.50
Red Khouribga	358.99±6.10	15.84±0.08	52.04±0.93	0.16±0.03	131.82±11.55
Tamellalet	467.22±3.79	36.96±0.34	-	0.16±0.02	104.71±6.24
Doukkala	394.9±7.40	18.28±1.48	-	0.16±0.02	112.51±9.83
Ras Elain	587.11±4.42	41.61±2.15	-	0.24±0.02	75.86±8.16
Ben Guerir	524.63±7.27	22.96±0.24	-	0.18±0.02	82.86±5.64
Ait Baamrane	643.66±3.25	51.33±4.10	-	0.24±0.02	52.48±6.17

But these values are less than those presented by Enza Maria Galati *et al*. (2003) (746 µg/ml of juice of whole fruits of Sicilian cultivars of prickly pear (*Opuntia ficus indica* (L.) Mill.) and Chang *et al.* (2008) (915µg/g GAE in methanol extracts of fruits of *Opuntia dillenii*).

The contents of betalains of cultivars (Table 2) were similar to those previously reported by El Gharras *et al.* (2008) (32.34 to 72.38 mg indicaxanthin/kg of juice) and Stintzing *et al.* (2003) (48.30 mg indicaxanthin/kg of juice) for Moroccan and Italian yellow prickly pear juice, respectively.

However, there were wide differences in terms of betaxanthins contents. The cultivars: *Ras Elain cv., Alkalaa cv. and Ait Baamrane cv.* contained the highest amounts (41.69mg/l - 51.33mg/l) followed by *Tamellalet cv.* and *Skhour Rhamna cv.* (36 to 37 mg/l) then *Ben Guerir cv., Ben Guerir cv.* and *Doukkala cv.* which contained the lowest amounts (< 23 mg/l). The red juice from *Khouribga* cv. contained (52.04, 15.84 mg/l) of betacyanins and betaxanthins respectively; on the other hand the yellow juices from the same origin contain only betaxanthins (26.68 mg/l).

Our results show that the contents of betalains were similar to those previously reported by El Gharras *et al.* (2008) (32.34 to 72.38 mg indicaxanthin/kg of juice) and Stintzing *et al.* (2003) (48.30 mg indicaxanthin/kg of juice) for Moroccan and Italian yellow prickly pear juice, respectively.

The Moroccan prickly pears juices contained more betaxanthins compared to values found by Chavez-Santoscoy *et al.* (2009) (3.1 to 189.9 mg indicaxanthin /kg of juice) and lower content than those found by Butera *et al.* (2002) (84.20 mg /kg of juice) in Mexican and Italian cultivars), respectively.

The effective concentrations (IC50) determined by DPPH radical-scavenging activity for juices varied between 52.48 ± 6.16 and 135.96 ± 12.5 µg/ml of juice (Table 2). The obtained values of DPPH were significantly different according to the varieties of cactus pear juices.

The juices from the nine cultivars were submitted to the ABTS radical cation decolourization assay. All juices had ABTS values in the narrow range of 0.16 to 0.24 mmol TE/g DW (Table 2) despite the significant differences in total phenols and betalains. The Moroccan prickly pear juices ABTS values were higher compared to those (4.20 to 5.31 µmol TE/g of edible pulp) of methanolic extracts from prickly pear fruit reported by Butera *et al.* (2002) and to values (17.4–25.8 mmol TE/L) obtained by Chavez-Santoscoy *et al.* (2009).

The correlation between phenolic content with antioxidant capacity of the juices of prickly pear is shown in Figure 4 and figure 5. The increase in phenolic content of the nine juices from different Moroccan areas was found to be linearly correlated with antioxidant capacity. The correlation coefficients between antioxidant capacity and total phenolic contents were R2=0.87 and R^2=0.96 for the ABTS and DPPH assays, respectively.

In general, correlation coefficients between antioxidant capacity and phenolic contents were positive and highly significant (P≤ 0.01) for the DPPH assay and significant (P≤ 0.05) for the ABTS assay. This observation agrees with the work of Veliogluo *et al.* (1998) regarding correlation of total phenolic content with antioxidant capacity in selected fruits, vegetables and grain products.

Correlation coefficients between antioxidant capacity and betaxanthins contents of the different cactus pear fruits juices are shown in figure 6 and figure 7. The correlation coefficients were r=0.613 and r=0.632 for the ABTS and DPPH assays, respectively. The increase in betaxhantins contents of the nine juices was found to be positively correlated with antioxidant capacity but less significantly than in the case of phenolics compounds, thus suggesting

that phenolics compounds contribute more significantly to the total antioxidant capacity than betalain pigments.

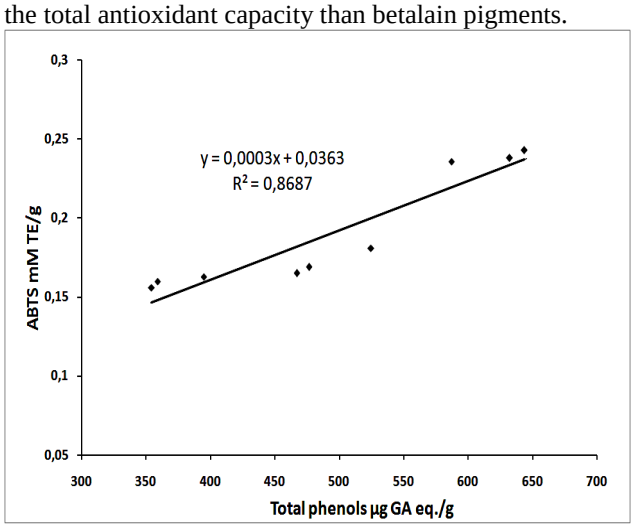

Fig 4: Correlations between antioxidant capacities (ABTS) and total phenolics of the prickly pears juices.

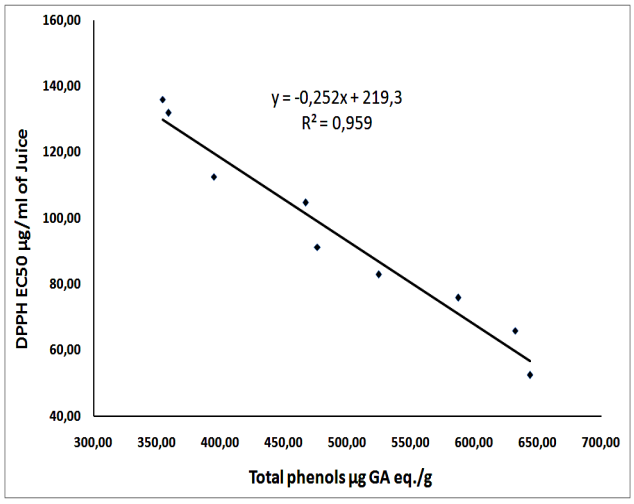

Fig 5: Correlations between antioxidant capacities (DPPH) and total phenolics of the prickly pears juices.

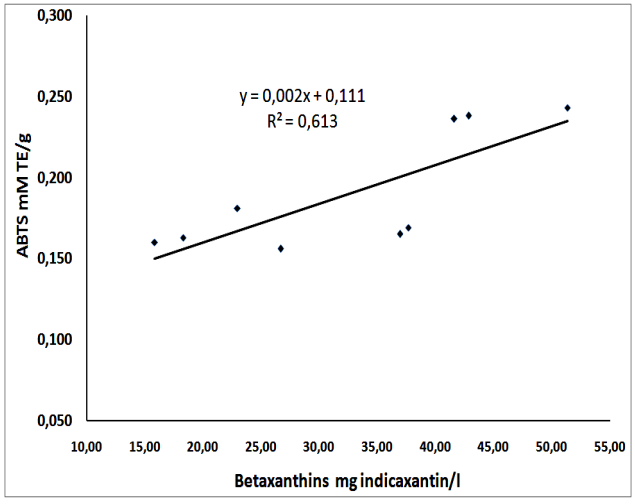

Fig 6: Correlations between antioxidant capacities (ABTS) and Betaxanthins content of the prickly pears juices.

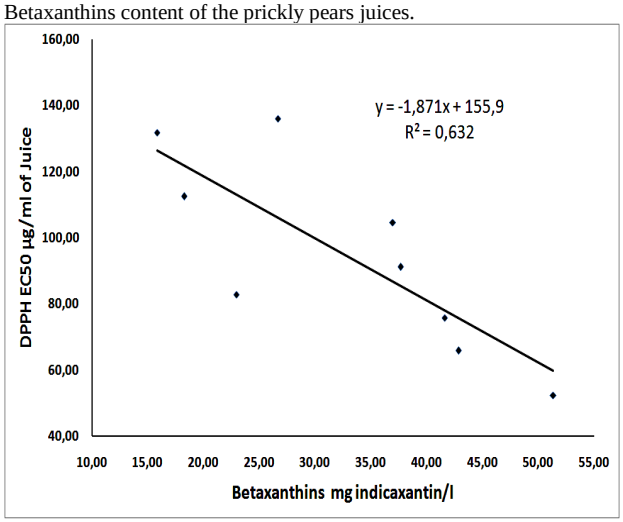

Fig 7: Correlations between antioxidant capacities (DPPH) and Betaxanthins content of the prickly pears juices**.**

4. Conclusion

This investigation shows the potential value of Opuntia cactus pear fruits as a good source of natural antioxidants. The high antioxidant capacity in juice cactus pears may be due to the high phenol contents or possibly a combination of individual antioxidants producing synergistic effects. The consumption of cactus pear fruit or its products may contribute substantial amounts of antioxidants to the diet.

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