Arabian Journal of Chemistry (2016) 9, S278–S281

King Saud University

Arabian Journal of Chemistry

www.ksu.edu.sa
www.sciencedirect.com

ORIGINAL ARTICLE

Extraction and determination of polyphenols and betalain pigments in the Moroccan Prickly pear fruits (*Opuntia ficus indica*)

Omar Khatabi [a,b,*], Hafida Hanine [a], Driss Elothmani [b], Aziz Hasib [c]

[a] *Laboratoire de Valorisation et Sécurité des Produits Agroalimentaires Fst Béni Mellal, Université Sultan Moulay Slimane, Morocco*
[b] *Laboratoire GRAPPE, Ecole Supérieure d'Agriculture d'Angers, France*
[c] *Laboratoire d'Environnement et Valorisation des Agro-Ressources, Fst Béni Mellal, Maroc, Université Sultan Moulay Slimane, Morocco*

Received 14 December 2010; accepted 11 April 2011
Available online 15 April 2011

KEYWORDS

Prickly pear;
Opuntia ficus indica;
Juice;
Antioxidants;
Polyphenols;
Betalains;
Betaxanthin;
Indicaxanthin;
Natural colorings

Abstract Polyphenols and betalain pigments respond property's antioxidants benefic for the human health (Will Strike et al., 2002; Lecerf, 1999). The item present shows procured results for strengths in polyphenols and in betalains to the time in fruits and in their juice for the period 2008–2009. Our searches show that rate in polyphenols is more increased in the entire fruit than in only the juice. We have equally remarked that the red prickly pear contains amounts of polyphenols more raised than those of the yellow variety.

This study has permitted us equally to value betalain pigments extracted from fruity juice. These are the betalains present in the epidermis and the pulp of the prickly pear confers on it its color varying from yellow to purple. Results show that yellow and red prickly pears contain imported strengths in betalains. Our work shows that the red prickly pear contains betaxanthin pigments in excess of the indicaxanthin that permits to valorize human's potential spring of genuine colorings. Betalains and polyphenols are antioxidants that contribute to nutritional prickly pears' quality and to their products of transformation.

* Corresponding author Address: ESA-GRAPPE, 55 rue Rabelais, BP 30748, 49007 Angers, France. Tel.: +212 654226404.
E-mail address: o.khatabi@groupe-esa.com (O. Khatabi).

Peer review under responsibility of King Saud University.

Production and hosting by Elsevier

http://dx.doi.org/10.1016/j.arabjc.2011.04.001
1878-5352

1. Introduction

The prickly pear (*Opuntia ficus indica*) is a tree originating in Mexico. It has the particularity to grow in arid semi-arid regions. It has been introduced in 16th century in several continents, notably in northern Africa. It yields an edible fruit called the prickly pear. In Morocco the prickly pear is realized for traditional uses as hedges of demarcation, to struggle against the erosion, for improvement of pastoral perimeters or else for the fruits consumption in expenses. Recently, in some countries as Mexico, Italy and South Africa, the Prickly pear is cultivated on considerable surfaces for industrial ends in Mexico, its culture stretches on a surface of 300,000 ha, while to Morocco, the surface of the culture of the Prickly pear is of about 120,000 ha (Boujghagh and Chajia, 2000; Arba, 2006) except Sahara and mountainous regions, the Prickly pear is broadly present in the Moroccan country, but its culture is badly organized and structured. Several searches show that the Prickly pear is a plant very rich in vitamins, minerals (Stintzing and Scheiber, 2003), amino acids and in sugars. It is used as foodstuffs, for medical applications, cosmetics and for production of the cochineal. Betalains concentrate in blooms and plants fruits of the generality of families Caryophyllals (Clement and Mabry, 1996). Those pigments are by-products of the betalamic acid. Betalains are pigments soluble in waters and are identified for the first time in the plant of the red beetroot (*Dull vulgaris*) (Wohlpart and Mabry, 1968).

The betalain pigments are in amaranth (*Amaranthaceae*) (Cai et al., 2005), bractées bougainvillaeas (*Nyctaginaceae*), and flowers (Minale et al., 1967) or other groups of plants in the Aizoaceae, Basellaceae, Didieraceae, Phytolaccaceae and Portulaceae (Glassgen et al., 1993; Schliemann et al., 1996; Gandia-Herrero et al., 2005). They are equally present in some mushroom kinds of the genus *Amanita* and *Hygrocybe* (Dopp and Musso, 1973). Betalains are a class of pigments, yellow Chromoes-alkaloids (betaxanthins). Some cultivars of the prickly pear are characterized by a deep red color caused blush by the presence of betacyanin pigments.

Several searchers have quantified and have identified betalains pigments in prickly pear of Mexican or Spanish origin (Castellanos-Santiago and Yahia, 2008; Fernandez-Lopez and Almela, 2001).

Other studies have shown that the juice of prickly pear is rich in polyphenols (Enza et al., 2003). Polyphenols have been studied for their protective effects against pathogenic bacteria and the virus infecting plants, or the radiance UV. As antioxidants, polyphenols have the property to trap free generated radicals continuously by our body or formed as a defense to attack on our environment (tobacco, pollution, infections, etc.) (Scalbert and Williamson, 2000; Arnaud et al., 2001). A long time set aside to conventional society, the fruit production of the prickly pear (*Opuntia ficus indica*) is in clean progress to see the socioeconomic importance an environmental of this culture. Those fruits are rich in C vitamin, pigments of specimen betalains, phenolic compounds, sugar reducers and inorganic salts (Maataoui and et Hilali, 2004). In this frame, the study itself is interested to estimate the amounts of total polyphenols and betalain pigments of different fruits and juice of Prickly pear of Moroccan origin.

2. Materials and methods

2.1. Samples

The plant material used in this study is composed of prickly pears from two different cultivars Moroccans: Ait Baamrane (Fig yellow variety Moussa) and Essaouira (Fig red crimson variety El Akri). The sampling was conducted between July and September 2008. All experiments were performed three times to ensure repeatability. Our results are expressed as mean values (see Fig. 1).

2.2. Total polyphenolic content

2.2.1. Extraction of polyphenols

The Prickly pear juice is prepared by grinding and centrifugation. The juice is then suspended in ethanol (75%) cold-containing metabisulfite sodium (0.5%). The mixture is kept stirring for 30 min at 4 °C, then filtered under vacuum. The filtrate obtained is taken up by ethanol (75%) cold-containing metabisulfite sodium (0.5%) for another extraction. The operation is repeated three times and the filtrates from these extractions are then extracted in alcoholic solution. In order not to cause any degradation of phenolic substances, alcoholic extract was evaporated under mild conditions (under vacuum at a temperature not exceeding a temperature of 35 °C) until an aqueous extract concentrated. To eliminate interference with no other polar substances depigmentation is done by acidifying the medium with metaphosphoric acid (2%) and adding 20% ammonium sulfate. The extract thus formed is depigmented by three extractions with half a volume of hexane. The phenolic compounds of the aqueous extract are depigmented three times with ethyl acetate (v/v). The organic phases obtained after three successive extractions are then combined. After vacuum evaporation of ethyl acetate, the dry residue obtained is taken up by a volume of methanol extract phenolic constituents, then purified. The latter is filtered through a membrane filter of 0.5 μm diameter pores, and then stored at −20 °C (Radi et al., 1997).

2.2.2. Determination of total polyphenols

The rate of total polyphenols is determined by the Folin–Ciocalteu method. This method is based on reducing properties of the phenolic function. Determination of the polyphenols was made as follows: an outlet for diluted phenolic extract was added 1.70 ml of water and 0.25 ml of Folin–Ciocalteu, 3 min later, 0.50 ml a 20% solution of sodium carbonate is added. The tubes are immediately added and incubated at a temperature of 40 °C for 30 min. The optical density is measured at a wavelength of 760 nm (Singleton and Rossi, 1965). Polyphenols are expressed as catechin equivalent.

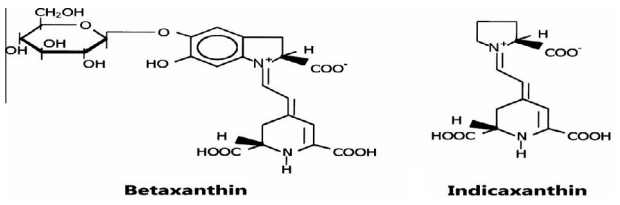

Figure 1 Structure of indicaxanthin and betaxanthin pigments.

2.3. Analysis of pigments betalains

The mixture of juice and methanol (1/5) (v/v) was homogenized by magnetic stirring. The mixture is then filtered through a nylon filter (0.45 μm). Finally, the value of the absorbance of methanolic extracts is obtained using a UV–Visible spectrophotometer (Shimadzu UV–Visible 160 A, Tokyo, Japan). The measurements were made using a quartz cell of 1 cm. The quantities of pigment extracts from fruit juices were evaluated by spectrophotometry according to Lambert Beer's law: $A = \log(I_{10}/I) = \varepsilon.L.c$, after measuring the absorbance at a languid wave corresponding to the maximum absorption of betaxanthin (482 nm) and indicaxanthin (532 nm). The molar extinction coefficients were respectively, of 62,000, $L\ mol^{-1}\ cm^{-1}$ for betaxanthin and 48,000 $L\ mol^{-1}\ cm^{-1}$ for indicaxanthin (Girod and Zryd, 1991). The results are expressed as mg pigment per kg of juice.

3. Results

The concentration of polyphenols (Table 1) varies depending on the nature of the sample analyzed (whole fruit or juice, red or yellow). The content of red polyphenols in prickly pear is about 15.34 ± 0.73 mg/kg of juice and 17.81 ± 0.10 mg/kg for whole fruit. In the yellow prickly pear the polyphenols content was 15.03 ± 1.36 mg/kg for juice and 15.03 ± 1.36 mg/kg for whole fruit. The average concentration of the polyphenol content of the whole fruit is higher than that of the juice alone, for the prickly pears red and yellow. This means that the pulp and seeds contain a quantity of polyphenols. The juice and whole fruit of red prickly pear contains a higher content of polyphenols compared with that of fig yellow.

Table 1 Content of total polyphenols in red prickly pear (El Akri) and yellow (Moussa).

Prickly pear	mg Catechin/kg
Red: whole fruit	17.81 ± 0.10
Red: juice	15.34 ± 0.73
Yellow: whole fruit	15.03 ± 1.36
Yellow: juice	7.76 ± 0.72

The determination of betalain pigments is done in the juice of both prickly pears, red and yellow. The absorbances were measured at the wavelength corresponding to maximum absorption of betaxanthin (482 nm) and indicaxanthin (532 nm). The first remark is the absence of red pigments, indicaxanthin absorbs at 532 nm in the yellow prickly pear. However, yellow pigments (betaxanthin) are present with a relatively large content of an average value of 37.822 mg/kg.

The mean level of the juice of betaxanthin prickly yellow is 37.822 ± 0.151 mg/kg. This value is similar to that found by Stintzing and Scheiber (2003) for Italian fruit. The content in the fruit is about 48.30 mg/kg of juice, lower than that found by Butera et al. (2002), which is 84.20 mg/kg of juice. Betaxanthin content found is much lower than that of the prickly pear juice from Spain, work reported by Fernandez-Lopez and Almela (2001) (Table 2).

Regarding the composition indicaxanthin yellow fruit juices, we see that levels vary widely between regions. We noticed that indicaxanthin is either absent or present in trace amounts. These results are consistent with the work of Fernandez-Lopez and Almela (2001) who mentioned the lack of juice in indicaxanthin yellow Prickly pear from Spain. In the case of Italian yellow fruit, Stintzing and Scheiber (2003) cited very low levels in indicaxanthin. The reported values are of the order of 1.30 mg/kg of juice. However, Butera et al. (2002) mentioned that indicaxanthin is present at levels of about 10.40 mg per 1 kg of juice (Table 2). In the juice of Prickly pear red, pigment analysis of betalains indicates the presence of two types of pigments: betaxanthin and indicaxanthin.

The average content of the juice of betaxanthin Prickly red analyzed is about 45.87 ± 0.123 mg/kg of juice. This value is slightly higher than that found by Stintzing and Scheiber (2003) for Italian fruit. These latter values are equal to 36.40 mg/kg of juice, which show that they are superior to those found by Butera et al. (2002) for the fruits of the same origin and which is close to 26.10 mg/kg of juice. The values found in this work are lower than those reported by Fernandez-Lopez and Almela (2001) which was about 300 mg/kg of juice (Table 3).

The average content in the juice of indicaxanthin prickly red analysis is about 56.53 ± 0.263 mg/kg of juice. This value is similar to that found by Butera et al. (2002) for Italian fruit (51.20 mg per 1 kg of juice), lower than that found by Stintzing

Table 2 Content and indicaxanthin betaxanthin (mg/kg juice) into the yellow prickly pear of different origins.

Origin	Indicaxanthin	Betaxanthin	References
Morocco (variety Moussa)	Absence	37.822 ± 0.151	This study
Italy	1.30	48.30	Stintzing and Scheiber (2003)
Italy	10.40	84.20	Butera et al. (2002)
Spain	Absence	250	Fernandez-Lopez and Almela (2001)

Table 3 Content and indicaxanthin betaxanthin (mg/kg juice) in the red prickly pear from different origins.

Origin	Indicaxanthin	Betaxanthin	References
Morocco (variety Moussa)	56.53 ± 0.263	45.87 ± 0.123	This study
Italy	51.20	26.10	Butera et al. (2002)
	73.90	36.40	Stintzing and Scheiber (2003)
Spain	190	300	Fernandez-Lopez and Almela (2001)

and Scheiber (2003) for the fruits of the same origin (73.90 mg/kg juice) and much lower than that cited by Fernandez-Lopez and Almela (2001) (190 mg per 1 kg of juice), corresponding to the fruit juice from Spain (250 mg/kg).

4. Conclusion

The extract of Moroccan prickly pear then presents an important source of poly-phenols. Our work shows that the levels of polyphenols are particularly higher in red fruits. These results also show that the red juice of *Opuntia ficus indica* is very rich in betalain pigments. This juice is characterized by a a higher content of red pigments in yellow pigments. The differences in results between regions are notably due to variability in the ecotypes cactus, physiology and growth conditions. Structural diversity in the sample of fig, makes it an exceptional product rich in natural antioxidants. The Moroccan prickly pear has also an important source of natural dyes that are in high demand in the food industry and trusted by consumers.

References

Arba, M., 2006. Dellahia a cactus pear cultivar from the Mediterranean coast of northern Morocco. Acta Horticult. 728, 37–41.

Arnaud, Basdevant, Martine, Laville, Eric, Lerebours, 2001. Traité de nutrition clinique de l'adulte. Flammarion Médecine-Sciences, pp. 165–177.

Boujghagh, M., Chajia, L., 2000. Genetic variation within different Cactus ecotypes of Moroccan origin. IVth internat. Congress Cactus Pear and Cochineal and the IVth general Meeting FAO-CACTUSNET. Hammamet, Tunisia.

Butera, D., Tesoriere, L., DI Gaudio, F., Bongiorno, A., Allegra, M., Pintaudi, A.M., Kohen, R., Livrea, M.A., 2002. Antioxidant activity of Sicilian prickly pear (*Opuntia ficus-indica*) fruit extracts and reducing properties of its betalains: betanin and indicaxanthin. J. Agri. Food Chem. 50, 689.

Cai, Y.Z., Sun, M., Corke, H., 2005. HPLC characterization of betalains from plants in the Amaranthaceous. J. Chromatogr. Sci. 43, 454–460.

Castellanos-Santiago, Elena, Yahia, Elhadi M., 2008. 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.

Clement, J.S., Mabry, T.J., 1996. Pigment evolution in the Caryophyllales: a systematic overview. Bot. Acta 109, 360–367.

Dopp, H., Musso, H. Die, 1973. konstitution dess Muscaflavins aus *Amanita muscaria* und uber betalaminsaure. Naturwissenschaften 60, 477–478.

Enza, Maria, Galati, Maria, Rita, Mondello, Daniele, Giuffrida, Giacomo, Dugo, Natalizia, Miceli, Simona, Pergolizzi, Maria, Fernanda, Taviano, 2003. Chemical Characterization and Biological Effects of Sicilian *Opuntia ficus indica* (L.) Mill. Fruit Juice: Antioxidant and Antiulcerogenic Activity. J. Agric. Food Chem., 51, 4903–4908, 490.

Gandia-Herrero, F., Escribano, J., García-Carmona, F., 2005. Betaxanthins as pigments responsible for visible fluorescence in flowers. Planta 222, 586–593.

Girod, PA., Zryd, JP., 1991. Biogenesis of betalains – purification and partial characterization of DOPA 4,5-dioxygenase from *Amanita muscaria*. Phytochemistry 30, 169–174.

Glassgen, W.E., Metzger, J.W., Heuer, S., Strack, D., 1993. Betacyanins from fruits of *Basella rubra*. Phytochemistry 33, 1525–1527.

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

Lecerf, J.M., (1999). Les antioxidants et les autres éléments protecteurs dans les jus de fruits et légumes - Publication de l'Union Nationale Interprofessionnelle des Jus de Fruits (UNIJUS), 30 Juillet 1999) Institute Pasteur – Lille. Available from: <www.unijus.org/juice/site/fo/unijus/publications.html>.

Maataoui, B.S., et Hilali, S., 2004. 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.

Minale, L., Piattelli, M., De Stefano, S., 1967. Pigments of Centrospermae VII. Betacyanins of Gomphrena globosa L.. Phytochemistry 6, 703–709.

Radi, M., Mahrouz, M., Jaouad, A., 1997. Etude des composés phénoliques de l'abricot en relation avec le phénomène de brunissement enzymatique. Thèse de doctorat à la Faculté des Sciences Semlalia de Marrakech (Maroc), p. 145.

Scalbert, A., Williamson, G., 2000. Dietary intake and bioavailability of polyphenols. J. Nutr. 130, 2073S–2085S.

Schliemann, W., Joy IV, R.W., Komamine, A., Metzger, J.W., Nimtz, M., Wray, V., Strack, D., 1996. Betacyanins from plants and cell cultures of *Phytolacca americana*. Phytochemistry 42, 1039–1046.

Singleton, V.L., Rossi, J.A., 1965. Colorimetry of total phenolics with phosphomolybdic-phosphotungstic acid reagents. Am. J. Enol. Vitic. 16, 144–158.

Stintzing, F.C., Scheiber, A., 2003. Evaluation of color properties and chemical quality parameters of cactus juices. Eur. Food Res. Technol. 216, 303–311.

Wohlpart, A., Mabry, TJ., 1968. On light requirement for betalain biogenesis. Plant Physiol. 43, 457–459.