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Health-promoting substances and antioxidant properties of *Opuntia sp.* fruits. Changes in bioactive-compound contents during ripening process

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ABSTRACT

Several *Opuntia* cactus fruits of different pulp colours from Argentina were studied at their physiological mature states. Analyses of these fruits showed very variable total soluble solid values and ascorbic acid contents ranging from 0.26 to 0.48 mg/g. Total phenolic compounds contents were between 0.54 and 1.2 mg of gallic acid/g, respectively. Purple *Opuntia spp.*, dark purple *Opuntia ficus-indica* and orange *Opuntia megacantha* presented the highest levels amongst the samples studied. The antioxidant activity of *Opuntia* fruits was very variable and presented vitamin C equivalent values (VCEAC) between 0.25 and 0.57 mg/g. Purple *Opuntia ficus-indica* showed the highest antiradical ability. Besides, the antioxidant activity, ascorbic acid and phenolic compound contents in yellow and orange *Opuntia megacantha* fruits were monitored in different stages during their ripening process. Concentration changes of betalains and chlorophylls comparing skin and pulp and other physicochemical parameters were also measured in these fruits.

1. Introduction

A great area of the world presents suitable soil and climate characteristics for the good development of cacti. Cactus fruits are true jewels of arid and semiarid climates. Their sweet and juicy pulp has interesting health-promoting properties attributed to the presence of certain bioactive compounds. Several epidemiological studies indicate that a diet rich in fruit and vegetables is related to lower incidences of cardiovascular diseases (Bazzano et al., 2002; Diplock, 1994) and some types of cancer (Ames, Shigena, & Hagen, 1993). These positive effects have been associated to the action of some antioxidant constituents of these natural foods. In the last decades, a great interest in the antioxidants and their role in the prevention of serious diseases has emerged worldwide. Cactus fruits are good sources of nutrients, vitamins and other bioactive constituents as polyphenols and pigments such as betalains (Kuti, 2004). Recent studies have reported about the antioxidant properties of betalains present in cactus fruits and have related them to the preventive action against several diseases and some other beneficial effects to health of consuming these foods (Tesoriere, Allegra, Butera, & Livrea, 2004). Betalains are also responsible for the fruit colours and, therefore, they have a strong influence in the consumer acceptance. These pigments are water-soluble nitrogenous compounds and they are also found in other plants as beetroot. According to their chemical structures, betalains are classified in two different groups, the betaxanthins, yellow and orange coloured substances, and the betacyanins, red to purple coloured ones (Hendry & Houghton, 1996).

The most widely spread cactus plants in Latin America are *Opuntia spp.* either cultivated or native. Amongst the main species of this genus, *Opuntia ficus-indica* species and its predecessor, *Opuntia megacantha*, are the most important from the agricultural point of view in this region. Their fruits present varied colours and are widely consumed by the local people. In order to characterise the main properties of these cactus fruits and their potential input to the diet, it is important to determine their contents of bioactive substances such as polyphenols, ascorbic acid and pigments at their mature state. Cactus pear fruits are non climacteric fruits and present a low respiration rate (Cantwell, 1995), hence, their nutrient concentration does not change considerably after harvest (Nazareno, Coria-Cayupán, Targa, & Ochoa, 2009). Therefore, the precise moment to collect them for marketing purposes, named commercial maturity, is close to the point in the fruit development defined as physiological maturity when the maximum growth has been achieved (Wills, Mc Glasson, Graham, & Joyce, 1998). The most apparent external signs of ripening are changes in colours,

Abbreviations: ARA, antiradical activity; Chl, chlorophylls; CM, commercial maturity; FC, Folin Ciocalteu; FF, fresh fruit; HPLC, high performance liquid chromatography; *Om*, *Opuntia megacantha*; TSS, total soluble solids; VCEAC, vitamin C equivalent antioxidant capacity.

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however, as far as we know, little research has been done on *Opuntia* fruit development concerning pigment content variations.

The main aims of this study were to determine the contents of these compounds responsible for health promoting properties such as pigments, polyphenols and ascorbic acid as well as the antioxidant activity of different *Opuntia sp.* fruits in their mature state. Besides, changes in the bioactive substance contents and the pigment compositions in fruit pulp and peel were determined at several development stages during the ripening process of two different colour *O. megacantha* fruits.

2. Materials and methods

2.1. Plant material

Fruits were collected in the province of Santiago del Estero located in the Argentinean region of the dry Chaco (27° 45′ S, 64° 18′ O, 170 metres over sea level). Different *Opuntia sp.* fruits were harvested at physiological maturity from cultivated plants. Ripening studies were performed during December, January and February, corresponding to the summer season. *O. megacantha* (yellow and orange) fruits were collected at random at five different development stages every 2 weeks since 4 weeks before physiological maturity until 4 weeks after that moment. The material studied corresponds to plants introduced by J. Ochoa in 1994 in Argentina from the Plant Research Nursery of the Centre Semi-Arid Forest Resources Texas A&M University (TX, USA). *Opuntia megacantha* orange pulp corresponds to clone number 1380 whilst *Opuntia megacantha* yellow pulp corresponds to clone number 1288.

The fruits were brought to the laboratory, then washed, dried and stored in refrigerated conditions until analysis. Total soluble solids (TSS) were measured from the filtered fruit juice using a thermo-compensated refractometer at 20 °C (Atago, Japan). The results, expressed as Brix values, are means obtained for not less than five fruits.

2.2. Methanolic extract preparation

For extract preparation, fresh fruits (FF) were peeled and 10 g representative of the edible portion of at least five fruits were homogenised in 30 ml of methanol, shaken for 3 min using a blender and filtered. The pellet was extracted twice again with 30 ml aliquots of methanol. The extracts were combined and methanol was added to constitute a total volume of 100 ml. Extracts were prepared in triplicate. In the case of peel analysis, 1 mm thickness of fruit skin was carefully separated by cutting it immediately before analysis. Remaining material was separated for pulp analysis.

2.3. Determination of antiradical activity (ARA)

Free radical scavenging ability of cactus pear extracts was measured using two bleaching methods: (i) the 1,1-diphenyl-2-picrylhydrazyl radical (DPPH$^{\cdot}$) method (Brand-Williams, Cuvelier, & Berset, 1995) and (ii) 2,2′-Azinobis-[3-ethylbenzothiazoline-6-sulphonic acid] radical cation (ABTS$^{+\cdot}$) method (Ozgen, Reese, Tulio, Scheerens, & Miller, 2006).

2.3.1. DPPH$^{\cdot}$ bleaching method

A 50 μl aliquot of the methanolic extract of fruits was placed in a cuvette, containing 3 ml of DPPH$^{\cdot}$ solution in methanol (initial absorbance at 515 nm c.a.1.00). The decrease in absorbance at 515 nm was determined by monitoring the absorbance changes in cycles of 30 s during 10 min using a spectrophotometer UNICAM UV2. All determinations were performed in triplicate for each extract. The percentage of the radical disappearance by the extracts was calculated according to the following equation:

$$\mathrm{ARA}\% = 100 \times (1 - A_{ss}/A_0) \quad (1)$$

where A_{SS} is the absorbance of the solution in a steady state and A_0 is the absorbance of DPPH$^{\cdot}$ solution before adding the antioxidant. The absorbance of the system at the steady state was estimated by mathematical fitting of kinetic curves performed with Origin 7.0 software. The calibration curve was prepared with ascorbic acid authentic sample in order to express ARA value as Vitamin C equivalent antioxidant capacity (VCEAC) in mg of vitamin C/g of fresh fruit as suggested by Kim, Lee, Lee, and Lee (2002).

2.3.2. ABTS$^{+\cdot}$ bleaching method

ABTS was dissolved in distilled water to yield a 7 mM solution. Radical cation solution was prepared by incubating the ABTS solution with a 2.45 mM potassium persulphate solution for 16 h in the dark at room temperature and subsequently diluted with water to a final absorbance of 1.00 ± 0.01 AU at 734 nm. For ARA determinations, 50 μl of fruit methanolic extracts were added to a cuvette containing 3 ml of the ABTS$^{+\cdot}$ solution. The decrease in absorbance at 734 nm was monitored in cycles of 30 s for 10 min using a spectrophotometer UNICAM UV2. All determinations were performed in triplicate for each extract. The percentage inhibition of ABTS$^{+\cdot}$ by the samples was calculated according to Eq. (1). A calibration curve was prepared with Trolox as a reference compound in order to express ARA value as Trolox Equivalent Antioxidant Capacity (TEAC) in mg Trolox/g FF.

2.4. Determination of ascorbic acid content

The quantitative analysis of ascorbic acid was performed by high performance liquid chromatography (HPLC). Fruit extracts were prepared immediately before analysis using a metaphosphoric acid–acetic acid mixture as extraction solvent. The determinations were carried out using a liquid chromatograph coupled with a diode array detector and a reverse phase SS WAKOSIL C18RS column (5 μm, 250 × 4.6 mm) at 24 ± 1 °C. The mobile phase used was pH 2.5 H_2SO_4 aqueous solution at 1 ml/min. The injection volume was 20 μl. The detection wavelength was 254 nm. Quantitative analysis was carried out by external standard method using pure ascorbic acid for calibration purposes.

2.5. Total phenolic content

It was determined from the methanolic extracts of the fruits by the Folin-Ciocalteu (FC) method according to Singleton and Rossi (1995) using the UV–Vis spectrophotometry measurement at 760 nm. Results were expressed taking gallic acid as reference compound in mg of this polyphenol/g FF.

2.5.1. Ascorbic acid correction

Not only phenolic compounds but also ascorbic acid contribute to the response of the FC assay, therefore, a correction was done by discounting ascorbic acid contribution previously determined by HPLC in total phenolic compound determination. In order to perform this correction, a calibration curve with ascorbic acid was also done and the final phenolic content value was recalculated (Asami, Hong, Barret, & Mitchell, 2003).

2.6. Spectrophotometric determination of pigments

Betalain contents were determined by preparing extracts from fruit pulps or peels using 80% aqueous methanol as solvent and measuring the absorption UV–Vis spectrum (200–700 nm). Pigment concentrations were calculated using their corresponding