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Original Article

Antioxidant activity in some red sweet pepper cultivars

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Abstract

Cultivars and growing conditions seem to play an important role in affecting the metabolism of antioxidant components and antioxidant capacity. Ten cultivars of red sweet peppers grown over two consecutive years were compared with regard to ascorbic acid, total reducing content, β-carotene, total antioxidant activity and free radical scavenging activity. Cultivar Flamingo had the highest ascorbic acid content followed by cultivars Bomby and Parker. All cultivars fulfilled 100% RDA requirement for vitamin C. Torkel and Mazurka excelled in terms of β-carotene. Flamingo had the highest total reducing content and antioxidant activity. There was no effect of harvest year on antioxidant activity; however, ascorbic acid, total reducing content (mainly phenolics) and β-carotene differed significantly. A weak correlation was observed between total reducing content and antioxidant activity as measured by ferric reducing antioxidant power (FRAP) and free radical (1,1-diphenyl-2-picrylhydrazyl, or DPPH) scavenging assays.

Keywords: Red sweet peppers; Genotypes; Antioxidants; FRAP; Total reducing content; Ascorbic acid

1. Introduction

Health concerns are driving plant breeders and food producers to develop plants and processed products with functional ingredients comprised of nutritive and non-nutritive antioxidants. Fruits and vegetables, which are rich in antioxidant molecules, are known for their health-promoting effects against degenerative diseases (Ames et al., 1993; Youdin and Joseph, 2001). In this context, it is relevant to know the type of health-promoting compounds present in the raw materials, and the concentration and total antioxidant activity governing their health-promoting effects (Kaur and Kapoor, 2001; Vander Sluis et al., 2001). Thus, it is of paramount importance to examine genotypes/cultivars for their antioxidant content and activity in order to find potential genotypes rich in antioxidants. Identification of the right genotype is of utmost importance for breeders and consumers alike. Such information could be incorporated into the breeding programmes for developing high-quality, antioxidant-rich genotypes, and it could be further exploited for producing high-quality processed products.

Red sweet pepper (*Capsicum annuum* L.) is a vegetable known for its rich antioxidant content. Fresh sweet peppers have exceptionally high ascorbic acid, a 100 g serving supplying 100% of the current RDA of 60 mg/day (Simmone et al., 1997). The attractive red colour is due to the various carotenoid pigments, which include β-carotene with pro-vitamin A activity and oxygenated carotenoids such as capsanthin, capsorubin and cryptocapsin, which are exclusive to this genus and are shown to be effective free radical scavengers (Matsufuji et al., 1998). Red peppers also contain moderate to high levels of neutral phenolics or flavonoids, namely quercetin, luteolin and capsaicinoids (Hasler, 1998). Capsicum

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cultivars have been identified as potential vegetables with high antioxidant activity (Ou et al., 2002). Wide variations exist among the different cultivars/genotypes of the same fruits and vegetables (Amakura et al., 2000; Cordensunsi et al., 2002; Raffo et al., 2002; George et al., 2004). Genetics seems to play a major role in the ability of cultivars to respond to biotic and abiotic stresses that are ultimately responsible for their overall phytochemical content and their antioxidant capacity. Red peppers—especially the pungent ones—have been extensively studied for their carotenoid content and other antioxidants (Gnayfeed et al., 2001; Horneo-Mendez et al., 2002; Russo and Howard, 2002; Topuz and Ozdemin, 2003). However, limited information is available on the antioxidant content and antioxidant activity of red sweet peppers. Keeping this in view, the main objective of the present study was to evaluate the antioxidant content and antioxidant activity in ten genotypes of red sweet peppers grown over two consecutive years.

2. Materials and methods

2.1. Fruit samples

Fruits of ten genotypes of sweet pepper (*C. annuum*) were grown during two consecutive years (2002–2003) in the green house of the Indo-Israel project at the Indian Agricultural Research Institute (IARI), New Delhi. The plants were raised in a semi-climate-controlled green house, where evaporative cooling was carried out with a pad and fan system. The temperature ranged from 16–30 °C during the growing season. Light intensity inside the green house during the winter months of December to mid-February was 300 μmol $m^{-2} s^{-1}$ because of the foggy weather conditions prevailing in New Delhi during that period. However, in the summer months light intensity was 500–600 μmol $m^{-2} s^{-1}$. Three replicates were taken for each cultivar, each comprised of three plants. Each replicate was composed of 1 kg of fruit from each cultivar, harvested at full red stage. Fruits were placed in polyethylene bags and transported under refrigerated conditions to the Division of Post-harvest Technology at IARI within 15 min. Samples received were stored at −80 °C until analyzed. Analysis was completed within a month of sample collection.

2.2. Determination of ascorbic acid

Ascorbic acid was quantitatively determined by using a relatively simple and fast 2,6-dichlorophenolindophenol-dye method as described by Jones and Hughes (1983) with slight modifications. Fresh samples (10 g) were homogenized with 10 mL of 3% metaphosphoric acid (volume-to-volume ratio) using a pinch of acid-washed quartz sand. The extract was made up to a volume of 100 mL and centrifuged at 3000*g* for 15 min at room temperature. Ten millilitres of supernatant were titrated against standard 2,6-dichlorophenolindophenol dye, which had already been standardized against standard ascorbic acid. Results were expressed as mg 100 g^{-1} on fresh weight basis.

2.3. Determination of carotenoids

2.3.1. Pigment extraction

The extraction of carotenoids was carried out according to the method described by Minguez-Mosquera and Horneo-Mendez (1993). A known weight (2 g) of sample was extracted with acetone using mortar and pestle. Extractions were repeated until complete exhaustion of colour was achieved (usually 4 or 5 extractions sufficed). All extractions were pooled in a separator and shaken with diethyl ether. A sufficient quantity of 10% NaCl was added at the end to aid in the separation of the phases; the aqueous phase was discarded. The organic phase was washed with 100 mL of anhydrous Na_2SO_4 (2%) solution to remove all the remaining water. It was saponified with 40 mL of 10% potassium hydroxide in methanol and shaken vigorously before being left in the dark for 1 h. After the addition of water, the pigments were subsequently extracted with diethyl ether, evaporated in a rotary evaporator and then made up to 25 mL with acetone. A 1 mL aliquot of this solution was centrifuged at 12,000 rpm and stored at −20 °C until analyzed. Losses occurring during the process were monitored with use of all-*trans* β-apo-8′-carotenal as the internal standard. All analysis was carried out in triplicate.

Separation and quantification of β-carotene was carried out using C-18 reverse phase column and binary gradient elution system (acetone-H_2O, 75:25) initially maintained for 5 min, changing linearly to 95:5 in 5 min, and maintained for 10 min. The flow rate was 1.5 mL/min, and the sample injection volume was 20 μL. At the end of the analysis, the column was washed with acetone for 3 min and conditioned with the initial proportion for 10 min. Detection of β-carotene was monitored at 450 nm. Final results were expressed as μg 100 g^{-1} fresh-weight capsicum tissue (Minguez-Mosquera and Horneo-Mendez, 1993).

2.4. Determination of total reducing content

Total reducing content refers to total phenolics and traces of other reducing agents such as ascorbic acid, etc. A 2 g sample was thoroughly crushed and homogenized in a mortar and pestle using 30 mL of 80% ethanol. The extract was placed in capped test tubes and heated at 60 °C in a water bath for 60 min. This step helps to complete the extraction of the phenolics as well as to destroy ascorbic acid to a large extent. The cooled extract was then centrifuged at 10,000*g* for 15 min at