Food Chemistry 132 (2012) 1161–1170

Contents lists available at SciVerse ScienceDirect

Food Chemistry

journal homepage: www.elsevier.com/locate/foodchem

Impact of boron, calcium and genetic factors on vitamin C, carotenoids, phenolic acids, anthocyanins and antioxidant capacity of carrots (*Daucus carota*)

Davinder P. Singh [a,b,1], Joel Beloy [a,b,1], Jennifer K. McInerney [a,c], Li Day [a,d,*]

[a] CSIRO Food Futures Flagship, North Ryde, Sydney, NSW 2113, Australia
[b] CSIRO Plant Industry, Private Mail Bag, Merbein, VIC 3505, Australia
[c] CSIRO Food and Nutritional Sciences, P.O. Box 10041, Gouger Street, Adelaide, SA 5000, Australia
[d] CSIRO Food and Nutritional Sciences, Werribee, Melbourne, VIC 3030, Australia

ARTICLE INFO

Article history:
Received 8 April 2011
Received in revised form 13 October 2011
Accepted 9 November 2011
Available online 23 November 2011

Keywords:
Carrot (*Daucus carota*)
Boron
Calcium
Carotene
Lycopene
Lutein
Phenolic acids
Vitamin C
Oxygen radical absorbance capacity (ORAC)
Cultivar difference
Minerals

ABSTRACT

Carrots (*Daucus carota* L.) were used to investigate the effects and interactions of cultivar and mineral supply on the nutritional quality (antioxidant potential, vitamin C, carotenoids and phenolic acids) of the resulting storage roots. The supplement of boron (B) and or calcium (Ca) in the feeding solutions, during plant growth, influenced the accumulation of other minerals, such as P, K, Mg, S and Na, in the storage roots ($p < 0.05$). When no additional B or Ca was supplied (e.g. −B or −Ca treatment), we observed 33–50% increase in the accumulated levels of α- and β-carotenes, and 45–70% increase of vitamin C. Carrots grown with no supplement of B in the nutrient solutions (e.g. −B treatment and −ve control) had significantly higher ($p < 0.001$) levels of total phenolic acids compared to the carrots with the supplement of B (e.g. −Ca treatment and +ve control). A strong positive correlation was observed between the total phenolic contents and ORAC values ($r = 0.932$) in all the cultivars. The results suggest that both cultivar and mineral supply were major determinants of nutritional quality of the carrots. The nutritional value of carrot crops (with an acceptable physical quality) can be enhanced by manipulating mineral nutrient applications.

1. Introduction

Vegetables are an important part of our diet. They provide, not only the major dietary fibre component of our food, but also a range of micronutrients, including minerals, vitamins and antioxidant compounds, such as carotenoids and polyphenols. The nutritional value of fruit and vegetables is often associated with their antioxidant capacities (Chu, Sun, Wu, & Liu, 2002; Vinson, Hao, Su, & Zubik, 1998). For example, carotenes act as a precursor for vitamin A, which can only be obtained from diet and which as an antioxidant, is known to play a role in the prevention of several diseases (Arscott & Tanumihardjo, 2010; Britton, 1995). Consumers are becoming increasingly aware of the nutrient quantity and composition of many essential minerals, vitamins and phytonutrients from fruit and vegetables, and the healthy benefit of regular intake of fruit and vegetables as part of the diet (Arscott & Tanumihardjo, 2010; Lindsay, 2000).

Mineral nutrients are essential for the growth, survival and reproductive success of plants (Epstein, 1965; Reid, 2001). Among the essential elements for healthy plants, boron (B) and calcium (Ca) are the two most important elements for supporting plant structural integrity and function of plasma membranes (Cakmak & Romheld, 1997; Cosgrove, 2005; Kirkby & Pilbeam, 1984). This is mainly because of their ability to interact with pectic polysaccharides in the plant cell wall matrix, thereby contributing to the maintenance of cell wall integrity and strength (Matoh & Kobayashi, 1998; Singh et al., 2010). Several studies have demonstrated that B application increases the yield of carrots and enhances drought tolerance of carrot cells whereas its deficiency results in small roots with yellow tops with a distinct white core in the middle (Demiray & Dereboylu, 2006; Gupta & Cutcliffe, 1985; Hole & Scaife, 1993). More recently, it has been shown that B deficiency leads to a decline in concentrations of other macronutrients, such as magnesium (Mg), Ca and potassium (K) or root nitrate content (Bolanos, Lukaszewski, Bonilla, & Blevins, 2004; Camacho-Cristobal, Rexach, & Gonzalez-Fontes, 2008; Gupta & Cutcliffe, 1985; Hole & Scaife, 1993), but accumulation of phenolics

* Corresponding author at: CSIRO Food Futures Flagship, North Ryde, Sydney, NSW 2113, Australia. Tel.: +61 3 9731 3233; fax: +61 3 9731 3250.
E-mail addresses: davinder.singh@dpi.vic.gov.au (D.P. Singh), joel.beloy@dpi.vic.gov.au (J. Beloy), jenny.mcinerney@csiro.au (J.K. McInerney), li.day@csiro.au (L. Day).
[1] Present address: Department of Primary Industries, Mildura, VIC 3502, Australia.

0308-8146/$ - see front matter Crown Copyright © 2011 Published by Elsevier Ltd. All rights reserved.
doi:10.1016/j.foodchem.2011.11.045

(Camacho-Cristobal, Lunar, Lafont, Baumert, & Gonzalez-Fontes, 2004; Hajiboland & Farhanghi, 2010; Mondy & Munshi, 1993). On the other hand, an increase in Ca concentration in the nutrient solution has been shown to have a negative impact on these phytonutrients and consequently causes a reduction in their antioxidant properties (Fanasca et al., 2006; Marin, Rubio, Martinez, & Gil, 2009; Paiva, Sampaio, & Martinez, 1998). The availability of minerals during plant growth also has a strong influence on concentrations of vitamin C, carotenoids, phenolic acids and overall antioxidant properties of fruit and vegetables (Camacho-Cristobal et al., 2004; De Pascale, Maggio, Fogliano, Ambrosino, & Ritieni, 2001; Fanasca et al., 2006; Flores, Navarro, Garrido, Rubio, & Martinez, 2004; Hajiboland & Farhanghi, 2010; Marin et al., 2009; Mondy & Munshi, 1993; Paiva et al., 1998).

Carrot (*Daucus carota*) is considered a primary vegetable in many countries. Diverse cultivars are grown in the world's temperate areas. In 2006, production worldwide was 24 million tonnes, with a market value exceeding US $2.0 billion (http://www.fao.org). The black, orange, purple, red and white colours in carrots originate from the anthocyanin and carotenoid pigments produced in these storage roots during maturation (Arscott & Tanumihardjo, 2010; Nicolle, Simon, Rock, Amouroux, & Remesy, 2004). Carrots contribute significantly to dietary vitamin A intake through the bioavailability of carotenes and, modestly, to other nutrients (Arscott & Tanumihardjo, 2010; Grassmann, Schnitzler, & Habegger, 2007; Sun, Simon, & Tanumihardjo, 2009). It is known that growth conditions and management practices affect the concentrations of these compounds in the storage roots of carrots which are important for both human nutrition and taste (Rosenfeld, Samuelsen, & Lea, 1998a, 1998b; Simon, Peterson, & Lindsay, 1982). It has been reported that the application of N fertilisers could modify the concentrations of carotenoids, vitamin C and total phenolic acid in the carrots storage roots (Smolen & Sady, 2009; Sorensen, 1999). However, there appears to be little information available regarding the effects of B and Ca on the nutritional status of carrots.

Considering that carrot is one of the primary vegetables in many countries and can adequately supply most, if not all, of the vitamin A daily requirement of human (from a 100 g serving of raw carrot (Arscott & Tanumihardjo, 2010), it is important to enhance the nutritional status of carrot where possible. This study was designed to investigate the impact of cultivar and mineral fertilizers (B and Ca) on the nutritional status of carrots in terms of the accumulation of vitamin C, carotenoids and total phenolic acids in the storage roots, and the effect of the accumulation of these phytonutrients on the enhancement of the antioxidant properties of carrots. Four treatments, in a combination of B and Ca application through controlled feeding regimes, were applied to the plants during their 3 month growing period.

2. Materials and methods

2.1. Plant material

Seeds of five commercial carrot cultivars namely 'Purple Dragon', 'Nutri-Red' (*D. carota* ssp. *sativus* var. *atrorubens* Alef), 'Kuettinger White' and 'Yellow', were supplied by Whatcom Seed Company and 'Kuroda' (orange) by Syngenta seeds Pty Ltd., Australia. Carrots were grown in a glasshouse maintained at 16–20 °C at Merbein, North West Victoria, Australia (34°12′49.83″S latitude, 142°02′40.14″E longitude), as described previously (Singh et al., 2010). Daily average integrated solar radiation during the growth period was 11.97 ± 1.04 megajoules per square metre ($MJ\,m^{-2}$) (Bureau of Meterology, Commonwealth of Australia-http://www.bom.gov.au). There were five seedlings of each cultivar per box and eight replicate boxes, per sowing. Each replicate box was 57 cm × 32 cm × 38.5 cm in size and seedling spacing was 10 cm. The layout of boxes in a glasshouse was fully randomised.

2.2. Treatments

The chemicals for preparing nutrient solutions were purchased from BDH (Poole, England). The seedlings were watered (1.0 L) with one of the nutrient solutions once a day and 0.8 L twice a day once the seedlings were 2–3 weeks old, to ensure a steady growth and to prevent root splitting. Four nutrient solutions were applied, as described previously (Singh et al., 2010). The positive control (+ve) solution contained 5.0 μM H_2BO_3, 3.0 mM $CaCl_2$, 0.5 mM NH_4NO_3, 0.4 mM KH_2PO_4, 0.7 mM K_2SO_4, 0.5 mM $MgSO_4$, 5.0 μM FeEDTA, 1.0 μM $MnSO_4$, 0.1 μM $ZnSO_4$, 0.1 μM $CuSO_4$, 0.5 μM Na_2MoO_4 and 0.02 μM $Co(NO_3)_2$. The negative control (−ve) solution was the above minus H_2BO_3 and $CaCl_2$; the minus B (−B) solution was minus H_2BO_3 and the minus Ca (−Ca) solution were all excluding $CaCl_2$. Tap water, used to make up feeding solutions, had low levels of B (45 μg/L) and Ca (10.8 mg/L).

Carrots were harvested 90–110 days after transplanting and washed 3–4 times in distilled water before sampling and storage. Each carrot was measured for fresh weight, length and diameter (largest). The roots were stored at −80 °C prior to further analyses.

2.3. Mineral analyses

At the time of harvest, carrot roots were slightly scrubbed with a brush in tap water to remove surface contaminants and washed in deionised water containing a few drops of domestic detergent. The carrots were then rinsed twice in deionised water before blot-drying on tissue papers. The washed carrot roots were diced into about 1–3 cm thickness and dried in an oven at 60 °C for 48–72 h. The samples were weighed before and after drying to determine the moisture loss. Minerals were extracted in triplicate from the carrot root sample according to the method described previously (Singh et al., 2010; Treeby & Storey, 2002). The dried material was ground in a Hammermill (POLYMIX-Micro-Hammermill MFC, Kinematica AG, Littau/Lucerne, Switzerland), using 1.5 mm mesh. The ground material (200 mg) for each sample was mixed in 2 ml of 15.2 M HNO_3 in an acid-washed test tube. The samples, after incubation overnight at the room temperature, were sequentially heated at 90 °C for 1 h and 143 °C for 4 h. The solutions were cooled and kept at 0 °C for 30 min. Millipore® water was added to the tube to make a final volume of 20 ml and this was vortexed for 1 min. Aliquots, of 10 ml each, were transferred into polyethylene tubes for mineral analyses. The concentrations of macro and trace elements were analysed by inductively coupled plasma-atomic emission spectrometry (Spectroflame ICP, Spectro Analytical Instruments, Kleve, Germany). Plant materials with known amounts of minerals were also analysed to verify both the acid extraction method and the accuracy and dependability of the results, as described previously (Singh et al., 2010; Treeby & Storey, 2002). For all analyses, median within-day and inter-day relative standard deviation (%RSD) values were from 5% to 10%. Standards were run to calibrate the ICP. The samples and standard solutions were matrix-matched. During the run, a blank and standard were run after every 10 samples to check the potential for drifting from the initial calibration. The calibration curves were re-normalised by a 2-point standardisation if a drift beyond ±6% of the set point was detected. Mineral results were then calculated from the calibration curve and expressed on a carrot fresh weight (FW) basis.

2.4. L-Ascorbic acid determination

Fresh carrot samples (5 g) were blended with 0.5 g of ice cold 30% metaphosphoric acid solution (MPA) to a homogeneous solution