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Evaluation of bioactive properties of Indian carrot (*Daucus carota* L.): A chemometric approach

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ABSTRACT

Sixteen Indian commercial carrot cultivars were analysed for variations in β-carotene, total phenolics, total flavonoids, total monomeric anthocyanin and antioxidant activity. Antioxidant activity was measured using four in vitro assays viz. ferric reducing antioxidant power (FRAP), cupric reducing antioxidant power (CUPRAC), 2, 2-diphenyl-1-picrylhydrazyl (DPPH) and Trolox equivalent antioxidant capacity assays (TEAC). Additionally six colour attributes were evaluated. Among carrot cultivars, significant differences ($p < 0.05$) were obtained with respect to antioxidant composition and antioxidant activity. Total phenols and total flavonoids varied from 7.98 to 291.48 mg/100 g fresh weight (fw) and 3.00 to 111.70 mg/100 g fw respectively. Chemometric tools like principal component analysis (PCA) and agglomerative hierarchical clustering (AHC) were applied to understand possible classification Indian carrot cultivars based on colour properties, bioactive antioxidant compounds and antioxidant potentiality. PCA revealed that the first two components represented 92.9% of the total variability in the total variation. AHC classified cultivars into four main groups on the basis of the measured parameters. Black coloured genotype was found to be rich source of phenols, flavonoids and anthocyanin with very high antioxidant activity. Orange cultivars were found to be rich sources for β-carotene compared to red & black cultivars.

1. Introduction

Reactive oxygen species (ROS) from internal as well as environmental sources have been implicated in the aetiology of various chronic human diseases like cancer, cardiovascular diseases, rheumatoid arthritis and age related macular degeneration (Halliwell, 1991). The effect of ROS can be balanced by natural dietary antioxidants and antioxidant enzyme (Alezandro et al., 2013). Thus, the consumption of fruits and vegetables is associated with reduced risk of these diseases as they are rich sources of natural antioxidants like vitamin C, tocopherol, carotenoids, phenolics and thiol antioxidants (Kaur & Kapoor, 2001; Boeing et al., 2012; Macedo et al., 2013).

Carrot is unique vegetable crop which belongs to apiaceae family rich in most of the natural antioxidants (Alasalvar et al., 2001; Nicolle et al., 2004; Grassmann et al., 2007; Sun et al., 2009). Cultivated carrot originated in Afghanistan region and being cultivated in China, USA, Russia, Uzbekistan, Ukraine, Turkey and India. Recently it is gaining high recognition and economic importance due to its high nutritional value and high concentration of natural antioxidants. Among vegetables, carrot is the single major source of β-carotene providing 17% of the total vitamin A consumption (Arscott & Tanumihardjo, 2010). Apart from β-carotene, root is good sources of various other lipophilic antioxidants like lycopene and lutein. The consumption of lutein is associated with prevention of age-related macular degeneration (Alves-Rodrigues & Shao, 2004) and reduced risk of atherosclerosis (Dwyer et al., 2001) whereas lycopene consumption is associated with reduced risk of certain type of cancer and cardiovascular diseases (Rao & Rao, 2007). It is also rich in hydrophilic phenolic antioxidants which are known for wide ranges of health promoting properties such as anticancer, anti-atherogenic, anti-inflammatory and antimicrobial.

In India carrot is popular root vegetable since ancient time and commercially it is grown mainly in Uttar Pradesh, Assam, Karnataka, Andhra Pradesh, Punjab and Haryana. Strong regional preference for specific coloured carrot has been observed in India. Red coloured carrot is typical to India (Leja et al., 2013) and predominantly cultivated in northern India for preparation of traditional sweet desert '*Halwa*'. Anthocyanin rich black carrot variety cultivated in northern India, used for preparation of traditional probiotic fermented beverage '*Kanji*'. Orange coloured carrot is mostly used as salad for domestic consumption and in fast-food corners along with red carrot. In south India only orange coloured variety is available which is used for preparation of curries. Traditionally red and black coloured carrot cultivated as local landrace maintained by farmers. Whereas orange coloured genotype of western origin are

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introduced to Indian market by private companies and research institutes. Realising the nutritional importance of natural antioxidant and other bioactive compounds present in carrot and overwhelming demand of consumer, various research institutes and private seed companies started breeding to develop high yielding bright coloured cultivars especially for red and black carrot. Many times carrot genotype of western origin is crossed with local genotypes to develop cultivars which acclimatized in Indian climatic condition in better way. As a result, many high yielding cultivars of carrot with different colours are available in Indian market. However, scanty information is available on level of antioxidant compounds and antioxidant potentiality of Indian cultivars. Detailed information on antioxidant properties of these cultivars with desired genetic architecture rich in nutraceutical content will serve as a reference material to develop nutraceutical rich carrot. Generation of such information will benefit both breeders as well as general consumer. In the present study various bioactive compounds, colour properties and antioxidant activity of sixteen commercial cultivars were investigated. Additionally principal component analysis (PCA) and agglomerative hierarchical clustering (AHC) were applied as chemometric tools to classify carrot cultivars based on levels of bioactive compounds, colour properties and antioxidant activity and to find relationship bioactive compounds, colour properties with antioxidant potentiality.

2. Materials and methods

2.1. Plant material

Sixteen commercial cultivars of carrot grown in the experimental fields of Indian Institute of Vegetable Research (IIVR), Varanasi, Uttar Pradesh, India were taken for the study. Crops were raised under optimal production practices. Roots were harvested at fresh harvest maturity stage. Roots were thoroughly washed with ordinary tap water to remove adhering soil and other dirt. After washing, roots were sorted out based on uniform colour and shape of the cultivars. A representative sample (500 g) was taken for subsequent analysis.

2.2. Extraction and analysis of anthocyanins

Carrot was minced with stainless steel knife. The amount of 150–250 g of minced carrot were homogenized after addition of ascorbic acid (5%, w/w) and water (25%, w/w) using a warring blander. An aliquot of 25 g the commuted carrot was extracted with 100 mL of methanol/0.1% HCl (v/v) for 2 h under dark condition. After centrifugation of the extract residue were re extracted with the same method. The combined supernatant were evaporated to dryness and re-dissolved in distilled water. The total monomeric anthocyanins content was determined on a UV–visible spectrophotometer by the pH-differential method (Wrolstad et al., 2005). The detection limit was 0.46 mg/L and the quantification limit was 1.41 mg/L, calculated from the residual standard deviation for the calibration curve and its slope.

2.3. Extraction and analysis of beta-carotene

Beta carotene was separated by column chromatography and quantified spectrophotometrically as described by Gayathri et al. (2004) with slight modification. The carrot sample of 100 g was repeatedly extracted with acetone using a pestle and mortar until the residue was colourless. The pooled acetone extracts were transferred into separatory funnel and the entire carotenoids were transferred to petroleum ethar phase. The pooled extracts were evaporated in vacuum at 30 °C and taken in petroleum ether. A column was prepared using absorbent for separation of β-carotene from extract. Columns of size 150 × 20 mm were packed with neutral aluminium oxide to a length of 10 cm and topped with a 1 cm layer of anhydrous Na_2SO_4. The column was washed with petroleum ether (60–80 °C, 25 mL). Ten millilitres of carotenoid extract was gently poured onto the column and the various fractions of pigments were eluted with the solvent systems in order of increasing polarity. The orange-coloured β-carotene band was eluted with petroleum ether (60–80 °C) containing 10% acetone. The eluent containing β-carotene was made up to a known volume and the concentration was measured in a spectrophotometer (SHIMADZU UV 1601) at 452 nm. The entire analysis was carried out in three replicates and the average values are reported on as-is basis.

2.4. Extraction of hydrophilic fraction

Water soluble free and bound phytochemicals of carrot were extracted according to the method reported previously (Chu et al., 2002) with slight modification. For the extraction of soluble free nutraceuticals, 5 g of the edible part of carrot was weighed and homogenized with 80% acetone (1:2 w/v) using a chilled warring blender for 5 min. The sample was then further homogenized using a polytron homogenizer for an additional 3 min to obtain a thoroughly homogenized sample. The homogenates were filtered through whatman no. 2 filter paper on a buchner funnel under vacuum. The residues were saved for extractions of bound phytochemicals. The filtrate was evaporated using a rotary evaporator under vacuum at 45 °C and reconstituted in 80% ethanol. The extracts were frozen at −40 °C until analysis.

For extraction of bound phytochemicals, the residues from above soluble free extraction were flushed with nitrogen gas and hydrolysed directly with 20 mL of 4 N NaOH at room temperature for 1 h with shaking. The mixture was acidified to pH 2 with concentrated hydrochloric acid and extracted six times with ethyl acetate. The ethyl acetate fraction was evaporated at 45 °C under vacuum to dryness. Phenolic compounds extracted by ethyl acetate were reconstituted in 10 mL of 80% ethanol and stored at −40 °C until analysis.

2.5. Determination of total phenolics and flavonoids content

Total phenol of carrot extract was estimated spectrophotometrically using folin ciocalteu reagent (Singleton et al., 1999). Aliquots (100 μL) of hydrophilic extract were mixed with 2.9 mL of deionized water, 0.5 mL of folin ciocalteu reagent and 2.0 mL of 20% Na_2CO_3 solution. The mixture was allowed to stand for 90 min and absorption was measured at 760 nm against a reagent blank in UV–vis spectrophotometer (SHIMADZU UV 1601). Results were expressed as gallic acid equivalent (mg GAE/100 g fw). Total flavonoids were analysed using aluminium chloride method (Zhishen et al., 1999). An aliquot (1 mL) of hydrophilic extract in 10 mL of volumetric flask containing 4 mL of distilled water, 0.3 mL portion of 5% $NaNO_2$ and 0.3 mL portion of 10% $AlCl_3$, $6H_2O$. The mixture was allowed to stand for 6 min at room temperature. Two millilitres of 1 mol NaOH was added and the solution was diluted to 10 mL with distilled water. The absorbance of the solution versus a blank at 510 nm was measured immediately. The results were expressed as catechin equivalent (mg CE/100 g fw).

2.6. Determination of antioxidant activity

Four in-vitro methods were used to analyses antioxidant activities. Free and bound hydrophilic fraction was pulled together for determination of hydrophilic antioxidant activity. Reducing power of carrot extracts were evaluated using FRAP and CUPRAC methods, whereas free radical scavenging assay were evaluated using DPPH and TEAC methods.

2.6.1. Ferric reducing antioxidant power (FRAP)

For the FRAP method (Benzie & Strain, 1996) the reagent was prepared by mixing 300 mM acetate buffer (pH 3.6), 10 mM TPTZ in 40 mmol HCl and 20 mmol $FeCl_3$ in the ratio 10:1:1 (v/v/v). Three millilitres of the FRAP reagent was mixed with 100 μL of aliquot in a test tube and vortexed in the incubator at 37 °C for 30 min in a water