PHYSICO-CHEMICAL AND ANTIOXIDANT EVALUATION OF DAUCUS CAROTA L.

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Keywords: Daucus carota, Physico-chemical, Phenolic content, Flavonoid content, Antioxidant evaluation

Abstract

Physico-chemical attributes and antioxidant potential of juice of different parts (whole carrot, cortex and inner core) of *Daucus carota* L. were comparatively evaluated. Total soluble solids and titratable acidity were found to be higher in whole carrot and lower in inner core. Total sugar content, chlorophyll a, chlorophyll b and carotenoids were found to be the highest in juice of inner core compared to the juice of cortex and whole carrot. Furthermore, total phenolic content and total antioxidant activities through reducing power assay, phosphomolybdenum assay and DPPH radical scavenging assay were found to be the highest in the juice of whole carrot whereas total flavonoid content was the highest in the juice of inner core. Comparatively, the juice of inner core exhibited higher antioxidant activities than that of cortex. The results suggest that the whole carrot exhibited good antioxidant properties.

Introduction

Fruits and vegetables play a significant role in reducing the risk of cancer, heart diseases and prevention of many diseases. This protective effect is due to the presence of health-promoting substances such as dietary fibers, minerals, vitamins and antioxidant compounds i.e. carotenoids, flavonoids, phenolic compounds and ascorbic acid (Jabbar *et al.* 2014).

Daucus carota L. (carrot) belongs to horticultural crops of high recognition with nutritional value due to the presence of high concentration of bioactive constituents i.e. vitamins (B1, B2, B6 and B12) and minerals. It is also a major source of carotenoids and depicts strong antioxidant potential due to the presence of ascorbic acid, anthocyanins, flavonoids and phenolic compounds, dietary glutathione and endogenous metabolites (Jabbar et al. 2014). Carrot carries other potentially beneficial health effects such as boasting antidiabetic, anticarcinogenic, antioxidant, anti-hypertensive, hepatoprotective, renoprotective, cholesterol and anti-inflammatory, cardiovascular disease lowering, wound healing activities and immune boosting activities. Carrots are characterized by a special fragrance, sweetness, crispiness and great variety of colours in the internal (inner core) and external (outer core or cortex) root tissues. It was reported that the cortex is a parenchymatic storage tissue with more flexible cells than those of core; this offers less rigid structure. On the other hand, inner core is mainly vascular although containing a few cortex-type cells radially arranged around its treachery elements. The ratio between cortex and core is an indication of differentiation of carrots, because the fraction of the core decreases during ripening in favour of the cortex (Nahimana et al. 2011). The quality of vegetables mostly depends on the components accumulated in fresh matrix, peel and cortex; therefore, it was very important to precisely estimate the quality of carrots.

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The aim of this study was to evaluate the physico-chemical attributes and antioxidant potential in juice of different parts of carrot.

Material and Methods

Folin-Ciocalteu's reagent, catechin and 2,2-diphenyl-1-picrylhydrazyl (DPPH) were purchased from Sigma-Aldrich (Switzerland). Ascorbic acid and gallic acid were purchased from Merck (Germany). All other chemicals and reagents were of analytical grade, purchased from Merck (Germany), Scharlau Spain (Spain) and Sigma-Aldrich (Switzerland).

Fresh red carrots were purchased from the local market of Karachi. They were cultivated in area of Khuzdar near Quetta (Pakistan). Carrots were washed to remove dirt and dried with cloth. Peel of the carrots was removed. Red part (cortex) and yellow part (inner core) of the carrots were separated. Juice of both weighed parts was extracted by using home juicer (Nova-multiple juice extractor, NJ-506, China). Juices were then filtered by cotton cloth, weighed and subjected to physico-chemical analysis.

Ten ml of the juice was extracted with 25 ml methanol containing 1% HCl, shaked and stood for 1 hr. Centrifuged the juice extract for 10 min at 3500 rpm. Supernatant was used for chlorophyll a, chlorophyll b, total carotenoid contents and antioxidant analysis.

Moisture content, ash content and titratable acidity (TA) of the juice of different parts of carrots were determined according to the A.O.A.C. official methods (1990).

pH of the juice was measured by using pH meter (Jenway 3510, England) and viscosity was measured using Ostwald viscometer. Density of the juice was determined using R.D. bottle whereas conductance was determined by conductivity meter (Jenway 4510, England).

Sugar content in the juice was determined according to previously reported colorimetric method (Okoye and Ugwu 2008). Total dissolved solids (TDS) of the juice were measured using TDS meter (S 518860, Korea) whereas total soluble solids (TSS) and refractive index of the juice were determined using refractometer (KRÜSS DR 6200, Germany).

Chlorophyll a, chlorophyll b and total carotenoids were determined using UV-visible spectrophotometer (Jenway 6300, England) (Dere *et al.* 1998). Absorbance of the sample was recorded at 400 - 700 nm on spectrophotometer. It was observed that chlorophyll a (C_a), chlorophyll b (C_b) and total carotenoids showed the maximum absorbance at 666, 653 and 470 nm, respectively; and the amount of these pigments was calculated.

Total phenolic content (TPC) of the juice was determined with some modifications according to Folin-Ciocalteu colorimetric method (Velioglu *et al.* 1998). The total phenolic contents were determined by comparing absorbance with those of standards with known gallic acid concentrations (50 - 500 mg/l concentrations, $r^2 = 0.982$).

Total flavonoid contents (TFC) of the juice were also determined (Zhishen *et al.* 1999). The total flavonoid contents were determined using catechin as a standard for calibration curve (20-200 mg/l, $r^2 = 0.993$).

Antioxidant capacity was carried out using reducing power assay (RPA) according to the method of Jayanthi and Lalitha (2011). Antioxidant capacity with respect to ascorbic acid in the juice sample was determined using calibration curve ($r^2 = 0.981$). Different concentrations of ascorbic acid (50 - 700 mg/l) were used to plot calibration curve.

The total antioxidant activity of the juice sample was also measured by phosphomolybdenum assay (PA) with some modifications (Prieto *et al.* 1999). The antioxidant activity was expressed relative to that of ascorbic acid. Standard curve of ascorbic acid was plotted at different concentrations (100 - 1000 mg/l).

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DPPH radical scavenging assay was also used to determine antioxidant activity (Yu *et al.* 2002). Per cent inhibition of DPPH radical by the juice sample was calculated.

Samples were prepared and analyzed in three replicates. Results were expressed as mean \pm SEM. All data were statistically analyzed by ANOVA using the software SPSS 21 with the DMRT to evaluate different parts of carrot (whole carrot, cortex and inner core) at level of significance (p ≤ 0.05).

Results and Discussion

Carrot is an enlarged flashy tap root consisting of the outer cortex and inner xylem core. The inner core was tougher and more fibrous than the outer one (cortex). This work was carried out to compare the physico-chemical attributes and antioxidant potential of juice of different parts of carrot. Carrot was having cortex of red colour and inner core of orange-yellow colour. Results are shown in Tables 1 and 2. The ANOVA indicated significant differences ($p \le 0.05$) among different parts of carrot regarding various parameters.

Total weight of whole carrot was 217.70 g in which weight of cortex and inner core were 131.62 and 86.08 g, respectively. Weight of extracted juice from whole carrot, cortex and inner core were 138.56, 80.59 and 57.97 g, respectively. Juice yield of any vegetable correlates with freshness of that vegetable. According to results, juice yield of whole carrot (yellow and red part) was 63.68%. Cortex and inner core contributed 36.98 and 26.70%, respectively. It was found that cortex has more contribution in the juice as compared to inner core.

The moisture content is an important constituent, and it is one of the physico-chemical characteristics, which decide the important sensory attributes like juiciness of sample. Moisture content of whole carrot was 90.47%. Relative contribution of cortex and inner core in the moisture content was 45.04 and 45.43%, respectively. This showed that inner core has relatively higher moisture content as compared to cortex. Inner core is the central part of carrot, comprises of xylem tissues, which are capable of water conducting and transport water from the soil to leaves for growth, that's why it contains higher amount of moisture content.

Ash content shows mineral content i.e. micro- and macronutrients. Ash content of juice of whole carrot was 0.703%. Ash contribution from cortex and inner core was 0.404 and 0.299%, respectively, showed that cortex has higher amount of ash content as compared to inner core.

pH shows the acidity or basicity of the juice. According to results, no significant difference was found in the pH of different parts of carrot. pH of the juice of whole carrot, cortex and inner core was found to be 6.42, 6.39 and 6.40, respectively. pH of all parts was high as compared to pH reported by Quitao-Teixeira *et al.* (2009), showing carrot was less acidic. pH correlates directly with the titratable acidity (TA). Titratable acidity shows the total amount of acids present in the juice. As pH, no significant difference was found in titratable acidity of the juices of whole carrot, cortex and inner core. From results, it was found that titratable acidity of whole carrot was 0.258 %. In contrast TA of cortex and inner core was 0.275 and 0.259%, respectively. TA of the juice of carrot was higher than TA of carrot as reported earlier (Sharma *et al.* 2009).

Viscosity shows the thickness of a fluid and density refers to the space among its particles. Viscosity and density of the juice of whole carrot were 0.105 milipoise and 1.035 g/ml, respectively. Comparatively, viscosity of the juice of cortex (0.107 milipoise) was higher than inner core (0.099 milipoise). Density was also found to be significantly higher in the cortex (1.040 g/ml) as compared to inner core (1.030 g/ml).

Conductance shows the presence of electrolytes (nutrients i.e. minerals, proteins, fatty acids and vitamins) in a juice. Conductance was significantly higher in cortex (8.83 mS). Conductance of the juice of whole carrot (8.80 mS) was significantly correlated with cortex while lower value

of conductance was found in inner core (7.82 mS). Conductance of the juice of all parts was relatively high to the findings as reported previously (Quitao-Teixeira *et al.* 2009). Cortex is comprised of phloem tissues, which are responsible for conducting the nutrients that's why cortex of the carrot showed greater conductance as compared to inner core. This research showed that cortex has high amount of nutrients (i.e. mineral content, proteins, fatty acids and vitamins etc.), which was also shown above through ash content. These nutrients are responsible for greater values of viscosity, density and conductance.

Sl. No.	Parameter		Unit	Whole carrot (Mean ± SEM)	Cortex (Mean ± SEM)	Inner core (Mean ± SEM)
1	Weight of carrot		g	$217.70 \pm 2.64^{\circ}$	131.62 ± 2.75^{b}	86.08 ± 1.71^a
2	Weight of juice		"	138.56 ± 4.89^{c}	80.59 ± 2.36^{b}	57.97 ± 1.88^{a}
3	Juice yield		%	$63.68 \pm 0.93^{\circ}$	$36.98 \pm 1.05^{\text{b}}$	26.70 ± 1.73^a
4	Moisture contents		"	90.47 ± 0.06^{b}	45.04 ± 0.03^a	45.43 ± 0.01^a
5	Ash contents		"	$0.703 \pm 0.00^{\circ}$	0.404 ± 0.01^{b}	0.299 ± 0.02^{a}
6	рН		-	6.42 ± 0.00^{a}	6.39 ± 0.01^a	6.40 ± 0.00^{a}
7	Titratable acidity		% citric acid	0.258 ± 0.06^a	0.275 ± 0.03^a	0.259 ± 0.03^{a}
8	Viscosity		milipoise	0.105 ± 0.00^{ab}	0.107 ± 0.00^{b}	0.099 ± 0.00^a
9	Density		g/ml	1.035 ± 0.00^{b}	1.040 ± 0.00^{c}	1.030 ± 0.00^a
10	Conductance		mS	8.80 ± 0.02^{b}	8.83 ± 0.01^{b}	7.82 ± 0.02^{a}
11	Total sugar contents		g glucose/100 ml	0.099 ± 0.001^{b}	0.104 ± 0.001^{b}	0.044 ± 0.001^{a}
12	TDS		g/l	5.34 ± 0.04^{b}	$5.33\pm0.01^{\text{b}}$	4.67 ± 0.01^a
13	TSS	Brix invert sugar	°Brix	7.65 ± 0.12^{b}	6.73 ± 0.02^a	6.71 ± 0.03^a
		Brix fructose	"	7.08 ± 0.01^{b}	6.85 ± 0.05^a	6.76 ± 0.04^a
		Brix sucrose	"	7.84 ± 0.05^{b}	6.67 ± 0.01^a	6.62 ± 0.05^a
		Brix glucose	"	6.96 ± 0.02^{b}	6.74 ± 0.01^a	6.72 ± 0.03^a
14	Refractive index		-	1.344 ± 0.00^{c}	1.343 ± 0.00^{b}	1.342 ± 0.00^{a}
15	Chlorophyll a		ppm	1.96 ± 0.00^{b}	0.22 ± 0.00^a	$2.26\pm0.01^{\text{c}}$
16	Chlorophyll b		"	2.93 ± 0.00^{b}	0.36 ± 0.02^a	$4.72\pm0.01^{\text{c}}$
17	Total c	arotenoids	"	207.66 ± 3.74^b	57.28 ± 1.45^a	277.70 ± 3.54^c

Table 1. Physico-chemical analysis of the juice of different parts of Daucus carota L. (carrot).

SEM = Standard error of the mean of triplicate analysis. Means with different superscripts lower case letters (a - c) in the same row are significantly different ($p \le 0.05$), were analyzed by ANOVA using DMRT.

Sugar content is the source of energy, and play an important role in flavor. This study indicated that cortex was significantly enriched with better amount of total sugar content (0.104 g glucose/100 ml) than inner core (0.044 g glucose/100 ml) and whole carrot (0.099 g glucose/100

ml). Total sugar content in the whole carrot significantly correlates with cortex, which is capable of storage of starch. So it has been cleared that cortex contains the high amount of sugar content (i.e. glucose units) (Baranska *et al.* 2005).

Total dissolved solids (TDS) is a measure of all organic and inorganic substances found in liquid in molecular, ionized or micro-granular (colloidal solution) suspended forms. TDS were almost same in the whole carrot (5.34 g/l) and cortex (5.30 g/l); and did not show any significant difference while inner core (4.67 g/l) showed the least amount of TDS.

S1.	Parameter	Unit	Whole carrot	Cortex	Inner core
No.			$(Mean \pm SEM)$	(Mean \pm SEM)	(Mean \pm SEM)
1	Total phenolic contents (TPC)	mg GAE/l	177.83 ± 2.57^{b}	108.50 ± 4.95^{a}	115.50 ± 4.95^{a}
2	Total flavonoid contents (TFC)	mg CE/l	86.33 ± 4.04^{b}	46.37 ± 5.62^a	$112.40 \pm 2.46^{\circ}$
3	Reducing power assay (RPA)	mg AA/l	45.27 ± 4.04^{b}	35.00 ± 0.40^a	36.40 ± 0.70^{a}
4	Phosphomolybdenum assay (PA)	mg AA/l	326.20 ± 0.99^{b}	252.00 ± 7.31^{a}	258.65 ± 9.41^{a}
5	DPPH radical scavenging assay	% inhibition	17.60 ± 0.49^{b}	12.39 ± 0.62^a	12.61 ± 0.62^{a}
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Table 2. Antioxidant analysis of the juice of different parts of Daucus carota L. (carrot).

GAE = Gallic acid equivalent, CE = Catechin equivalent, AA = Ascorbic acid, SEM = Standard error of the mean of triplicate analysis. Means with different superscript lower case letters (a - c) in the same row are significantly different ($p \le 0.05$), analyzed by ANOVA using DMRT.

TSS in the vegetable juices has shown the carbohydrates, organic acids, proteins, fats and total dissolved solids. Total soluble solids (Brix glucose, Brix sucrose, Brix fructose and Brix invert sugar) were found to be higher in the whole carrot (6.96-7.84 Brix). It was found that cortex and inner core were significantly correlated to each other.

From results, it was shown that refractive index of the juice was directly affected by TDS. Refractive index was found to be slightly higher in the whole carrot (1.344) as compared to cortex (1.343) and inner core (1.342).

Chlorophylls (a and b) are fat soluble pigments found in the vegetables and fruits. Chlorophylls a and b in the fruits and vegetables are found in ratio of the 3 : 1. Chlorophyll a (2.26 ppm) and chlorophyll b (4.72 ppm) were found to be higher in the inner core of carrot. The lowest amount of chlorophyll a (0.22 ppm) and chlorophyll b (0.36 ppm) were found in the cortex of carrot.

Carrot is the richest source of carotenoids. Red-orange colour of carrot is due to the presence of carotenoid pigments in carrot. β -carotene and α -carotene are fat soluble pigments responsible for orange colour. Most carrot carotenoids are polyunsaturated and highly reactive (Nahimana *et al.* 2011). The carotenoid ability of quenching singlet oxygen confers them significant antioxidant properties that are relevant in the prevention of several human diseases (Gonzalvez *et al.* 2014). Total carotenoid content was also found to be higher in the inner core (277.70 ppm) than cortex (57.28 ppm) while total carotenoid content in the whole carrot was 207.66 ppm. It showed that

inner core accumulated more carotenoids. Results showed similar trend of carotenoids as reported earlier (Nahimana *et al.* 2011, Gonzalvez *et al.* 2014). It was also showed that inner core has major contribution for chlorophyll a, chlorophyll b and carotenoid contents. Unequal distribution of carotenoids between the two tissues as discussed earlier confers better colour to the cortex (Gonzalvez *et al.* 2014). The colour of the inner core was paler than the cortex due to higher carotene content. Generally, the carotene concentration correlates with an orange-yellow colour.

Table 2 shows the total phenolic content (TPC), total flavonoid content (TFC) and antioxidant activity using reducing power (RPA), phosphomolybdenum (PA) and DPPH free radical scavenging assays.

Polyphenols have received considerable attention because of their physiological functions including antioxidant, antimutagenic and antitumor activities. They have been reported to be a reducing agent and potential contender to combat free radicals, which are harmful to human body and food systems (Sharma *et al.* 2012, Kaur and Kapoor 2002). The antioxidant activity of phenolic compounds is mainly due to their redox properties, which can play an important role in absorbing and neutralizing free radicals, quenching singlet and triplet oxygen or decomposing peroxides (Yen *et al.* 2008). More specifically, results illustrated that total phenolic content (TPC) was found to be higher in the whole carrot (177.83 mg GAE/l) in comparison with inner core (115.50 mg GAE/l) and cortex (108.50 mg GAE/l). TPC of cortex and inner core was significantly correlated. Hence it was cleared that the whole carrot has more ability to scavenge free radicals than inner core and cortex. Flavonoids are highly effective scavengers for various oxidizing molecules including singlet oxygen and various free radicals. Total flavonoid content (TFC) in carrots was found to be higher in the inner core (112.40 mg CE/l). TFC in the whole carrot and cortex was found to be 86.33 and 46.37 mg CE/l, respectively.

Total antioxidant activity of the juice is connected with the amount of bioactive compounds like phenolic acids, flavonoids, carotenoids and vitamins (Jabbar *et al.* 2014). The antioxidant activity of the juice of different parts of carrot was evaluated using three different assays including reducing power (RPA), phosphomolybdenum (PA) and 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging assays. The results are shown in Table 2.

Antioxidant activity was evaluated using oxidation-reduction method through RPA and PA. Antioxidant activity through reducing power assay shows the conversion of Fe^{+3} to Fe^{+2} . Compounds with reducing power indicate that they are electron donors, and have ability to reduce the oxidized intermediates, which are formed as a result of lipid peroxidation processes (Yen and Chen 1995). In case of PA, molybdenum [Mo (VI)] in the presence of antioxidant compounds is reduced to form green phosphate [Mo (V)] complex (Prieto *et al.* 1999). RPA and PA exhibited the highest antioxidant activities 45.27 and 326.20 mg ascorbic acid/l, respectively in the whole carrot. In comparison, inner core had greater antioxidant activities than cortex.

DPPH free radical scavenging assay was also used to evaluate the antioxidant activity. DPPH radical at its maximum wavelength 517 nm can easily receive an electron or hydrogen from antioxidant molecules to become a stable diamagnetic molecule. Owing to the DPPH radical's ability to bind H, it is considered to have a radical scavenging property. In presence of antioxidant, DPPH radical is converted into DPPH-H (diphenylhydrazine). Discoloration of solution occurs due to decrease of DPPH radicals (Aksoy *et al.* 2013). DPPH assay showed the highest antioxidant activity in the whole carrot (17.60%). It was also observed that antioxidant activity of the inner core (12.61%) was higher than cortex (12.39%). It had been cleared that all assays showed same order of antioxidant capacity as compared to cortex and inner core. These parts contributed individually, and exhibited the highest antioxidant activity in the whole carrot. Antioxidant

activities using all these assays also showed correlation with TPC. Comparatively, juice of the inner core had good antioxidant activity regarding the ability to scavenge free radicals than cortex. Hence it was confirmed that the inner core had major contribution in antioxidant activity of whole carrot.

Conclusions

This analysis of different parts of carrot described that the juice of whole carrot (both cortex and inner core) had the best quality with respect to physico-chemical attributes and antioxidant capacity rather than the juice of cortex and inner core. On comparing the juices of the cortex and inner core, juice of inner core had good quality with respect to antioxidant analysis and other nutritional facts than the cortex. Inner core contained high amount of carotenoids and total antioxidant activity, so it had major contribution in the juice of whole carrot.

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(Manuscript received on 18 August, 2016; revised on 2 October, 2016)