Phenolic compounds and their antioxidant properties in different tissues of carrots (*Daucus carota* L.)

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Received 5 October 2003, accepted 9 January 2004.

Abstract

Phenolic compounds, their antioxidant properties and distribution in carrots were investigated in this study. Carrots contained mainly hydroxycinnamic acids and derivatives. Among them chlorogenic acid was a major hydroxycinnamic acid, representing from 42.2% to 61.8% of total phenolic compounds detected in different carrot tissues. The phenolic contents in different tissues decreased in the following order: peel > phloem > xylem. Although carrot peel accounted for only 11.0% of the amount of the carrot fresh weight, it could provide 54.1% of the amount of total phenolics in 100 g fresh weight of carrots, while the phloem tissue provides 39.5% and the xylem tissue provides only 6.4%. Antioxidant and radical scavenging activities in different tissues decreased in the same order as phenolic content and correlated well with total phenolic contents. All phenolic extracts had stronger radical scavenging ability than pure chlorogenic acid, vitamin C and β-carotene. Therefore, we suggest that phenolics could play an important role in antioxidant properties in carrots and other hydroxycinnamic derivatives such as dicaffeoylquinic acids in the extracts may exert some strong antioxidant activities along with chlorogenic acid.

Key words: carrot, phenolic compounds, antioxidant activity, free radical scavenging activity.

Introduction

The consumption of fruits and vegetables has been associated with low incidences and mortality rates of many diseases, particularly cancer [1, 2, 3] and cardiovascular disease [4]. Eating fruits and vegetables also reduces blood pressure, boosts the immune system, detoxifies contaminants and pollutants, and reduces inflammation [5, 6]. The protection that fruits and vegetables provide against diseases has been attributed to the various antioxidants contained in them [7, 8, 9, 10].

Fruits and vegetables are good sources of natural antioxidants, containing many different antioxidant components [11, 12, 13]. These antioxidants include carotenoids, vitamins, phenolic compounds, flavonoids, dietary glutathione, and endogenous metabolites [14], having been shown to function as singlet and triplet oxygen quenchers, free radical scavengers, peroxide decomposers, enzyme inhibitors, and synergists [14]. Phenolic compounds are found in most fruits and vegetables. They have been reported to possess antioxidant activity which allows them to scavenge both active oxygen species and electrophiles, to inhibit nitrosation and to chelate metal ions, to have the potential for autoxidation and the capability to modulate certain cellular enzyme activities [15]. The majority of the antioxidant activity of fruits and vegetables may be from phenolic compounds rather than vitamin C and E, or β-carotene since some phenolic compounds have much stronger antioxidant activities against peroxyl radicals [16].

Carrots have been ranked sixth in per capita consumption among 22 popular vegetables [17]. They are being increasingly consumed with increasing use of minimally processed vegetables. Carrots are the major source of provitamin A, providing 17% of the total vitamin A consumption and also rich in α- and β- carotenes [18]. Carotenes have been proved to possess antioxidant activity. However, little is known about phenolic compounds in carrots and their antioxidant properties. Therefore, the objective of this research was to evaluate phenolic compounds, their antioxidant properties and distribution in different tissues of carrots so as to provide the information helpful for the minimal processing and the utilization of the processing by-products such as carrot peel.

Materials and Methods

Plant material and extraction: Carrots (*Daucus carota* L.) were obtained from a local wholesale market. Two varieties of carrots (Chibagosun and Hitomigosun) were investigated in this study. Peel, phloem and xylem tissues of carrots were separated with a peeler and knife and weighed respectively to calculate their proportion. 10 g of peel, phloem and xylem tissues were sampled respectively, cut to small pieces and homogenized in 15 ml of acetone and extracted for 60 min. The homogenate was centrifuged at 7,000×g for 20 min. The supernatant was collected and the residue was added with 20 ml of 60% acetone for two successive re-extractions. The collected supernatants were combined and the residue was discarded. Acetone was removed from the supernatant by evaporation under vacuum at 35ºC, pigments and fatty acids were eliminated by two successive extractions with petroleum ether (2:1, v:v). The aqueous phase was collected as a crude extract.

Determination of phenolic compounds: The crude extract was acidified to pH 2.0 and loaded into a Sep-Pak C18 cartridge

preconditioned with methanol and acidic water. After washing with distilled water, the Sep-Pak C18 cartridge was eluted with methanol. The methanol was removed under vacuum at 35ºC. The remaining aqueous phase was made up to 10 ml and filtered with 0.45µm Advantec filter for determination of total phenolics, HPLC analysis of phenolic compounds, and antioxidant properties. Total phenolic content of each extract was determined by the Folin-Ciocalteu reagent as described by Singleton and Rossi [19] using gallic acid as a standard and expressed as mg gallic acid equivalent (GAE)/100 g fresh weight. Individual phenolic compounds were separated on a Luna 5u C18 column (150×4.60 mm from Phenomenex) by HPLC (Shimadzu LC-6AD Liquid Chromatograph equipped with Shimadzu SPD-M10Avp Diode Array Detector). Mobile phase was A: acidic water (0.1% phosphoric acid) and B: acetonitrile-phosphoric acid (0.1% phosphoric acid). The flow rate was 1.0 ml/min. The best separation was obtained at 40ºC using the following gradient elution: 5% B at 0 min and 50% B at 30 min. Identification of phenolic compounds was achieved by their retention time and UV spectra recorded with Shimadzu SPD-M10Avp diode array detector in comparison with authentic chlorogenic acid, caffeic acid and phenolic compounds identified in green coffee bean extract and carrots according to the methods of Clifford [20] and Babic et al. [21]. The contents of phenolic compounds were expressed in mg per 100 g fresh weight equivalent chlorogenic acid for hydroxycinnamic acids except caffeic acid.

Antioxidant activity: Evaluation of antioxidant activity based on coupled oxidation of β-carotene and linoleic acid was conducted as described by Taga et al. [22] with some modifications. β-carotene (6 mg) was dissolved in 20 ml of chloroform. A 3 ml of the solution was added to a conical flask with 40 µl linoleic acid and 400 µl Tween 20. Chloroform was removed with a rotary evaporator under vacuum at 35ºC. Oxygenated distilled water (100 ml) was added to the β-carotene emulsion and mixed well. 3 ml aliquot of the β-carotene emulsion and 0.2 ml of the diluted extract were placed in a test tube and mixed well. The tubes were immediately placed in a water bath and incubated at 50ºC. Oxidation of β-carotene emulsion was monitored spectrophotometrically by measuring absorbance at 470 nm. Sample absorbance was measured at 10, 20, 30, 40, 50, and 60 min after incubation. A control consisted of 0.2 ml distilled water, instead of the extract. The degradation rate of the extracts was calculated by first order kinetics:

Sample degradation rate = ln (a/b) ×1/t

where: ln = natural log; a = initial absorbance at time 0; b = absorbance at 10, 20, 30, 40, 50 and 60 min; t = time (min). Antioxidant activity (AA) was expressed as % inhibition relative to the control using the equation:

$$AA(\%) = \frac{\text{Degradation of control} - \text{Degradation of sample}}{\text{Degradation of control}} \times 100$$

Free radical scavenging activity: Free radical scavenging activity of the extracts was measured using the method of Brand-Williams et al. [23] with some modification. A 0.1mM solution of DPPH (1,1-diphenyl-2-picryl-hydrazyl) in methanol was prepared and 4 ml of this solution was added with 0.2 ml of the extract. The decrease in absorbance at 517 nm was measured at 60 min. A control was added with 0.2 ml of distilled water instead of the extract. Free radical scavenging activity was expressed as the percentage of DPPH decrease. The IC50 value, defined as the amount of antioxidant necessary to decrease the initial DPPH concentration by 50%, was calculated from the results and used for comparison of the quality of antioxidant extracts. The radical scavenging activities of vitamin C, chlorogenic acid and β-carotene were also measured in the same condition for comparison purposes.

Data handling: Three duplicate experiments were carried out in this study. Values represent the means from three duplicate experiments.

Results and Discussion

Phenolic composition and total phenolic content in different tissues of carrots: Individual phenolic compounds in carrots were analyzed by HPLC method. Carrots contained mainly hydroxycinnamic acids and derivatives such as chlorogenic acid, caffeic acid, 3'-caffeoylquinic acid, 4'p-coumaroylquinic acid, 3',4'-dicaffeoylquinic acid, 3',5'-dicaffeoylquinic acid and some unidentified hydroxycinnamic derivatives (Figure 1). Unknown peak 1 had a spectrum different to hydroxycinnamic derivatives (Figure 2), which was not detected in the study of Babic et al. [21]. Unknown peaks 7, 8, 9, 10 and 11 had similar spectra to hydroxycinnamic acids (Figure 2). They could be hydroxycinnamic derivatives. The isocoumarins 6-hydroxymellein and 6-methoxymellein, previously identified by Harding and Heale [24], were not detected in this study. pHB, identified by Babic et al. [21], was also not detected. The results show that phenolic composition in different tissues was similar, but the content of individual phenolic compounds varied. The contents of phenolic compounds in different carrot tissues are shown in Table 1. Hydroxycinnamic acids and their derivatives were major phenolic compounds in the carrots, representing 99.7% (in Chibagosun) and 98.7% (in Hitomigosun) of total phenolics detected in peel, 96.5% (in Chibagosun) and 91.0% (in Hitomigosun) in phloem, and 75.6% (in Chibagosun) and 73.7% (in Hitomigosun) in xylem. Among them, chlorogenic acid (5'-caffeoylquinic acid) was a major hydroxycinnamic acid in carrot tissues, representing from 42.2% to 61.8% of total phenolic compounds detected in different carrot tissues. The phenolic contents detected in different tissues decreased in the following order: peel > phloem > xylem.

Total phenolics of the extracts were also determined by the Folin-Ciocalteu assay (Figure 3). In the Chibagosun carrot, total phenolics in peel were 78.3 mg GAE/100 g fresh weight, 8.3 and 19.1 times higher than in phloem and xylem tissues respectively. In the Hitomigosun carrot, total phenolics in peel were 62.0 mg GAE/100 g fresh weight, 3.2 and 9.5 times higher than in phloem and xylem tissues. Kähkönen et al. [25] found that carrot peel and flesh contained 6.6 and 0.6 mg gallic acid equivalent/g dry weight. Vinson et al. [26] found in carrots 46.4 mg catechin equivalent/100 g fresh weight. The different values in the literature may be due to different extraction methods and the ways to express the results. In addition, different cultivars of carrots could cause the differences as seen the differences between two cultivars tested in this study. The phenolic contents

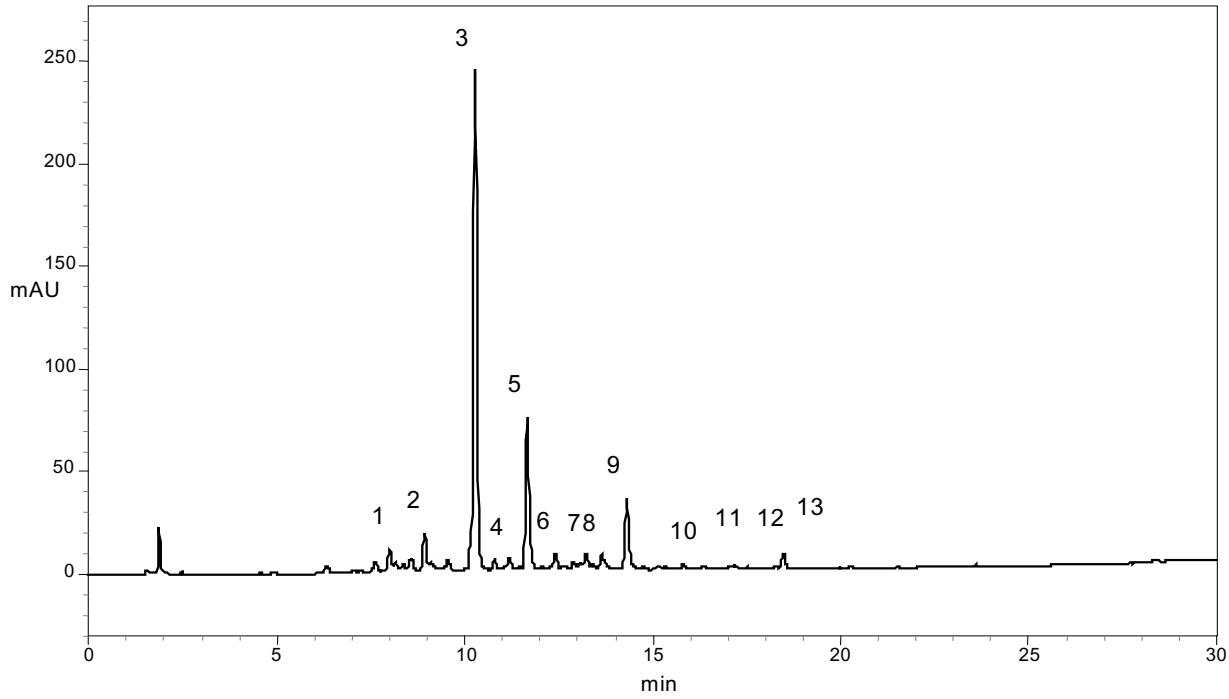

Figure.1. Typical HPLC chromatograph of phenolic compounds in carrots at 280 nm. Peak identification: (1) unknown; (2) 3'-caffeoylquinic acid; (3) 5'-caffeoylquinic acid; (4) caffeic acid; (5) cis-5'-caffeoylquinic acid; (6) 4'p-coumaroylquinic acid; (7) unknown hydroxycinnamic derivative; (8) unknown hydroxycinnamic derivative; (9) unknown hydroxycinnamic derivative; (10) unknown hydroxycinnamic derivative; (11) unknown hydroxycinnamic derivative; (12) 3'4'-dicafferoylquinic acid; (13) 3'5'-dicafferoylquinic acid.

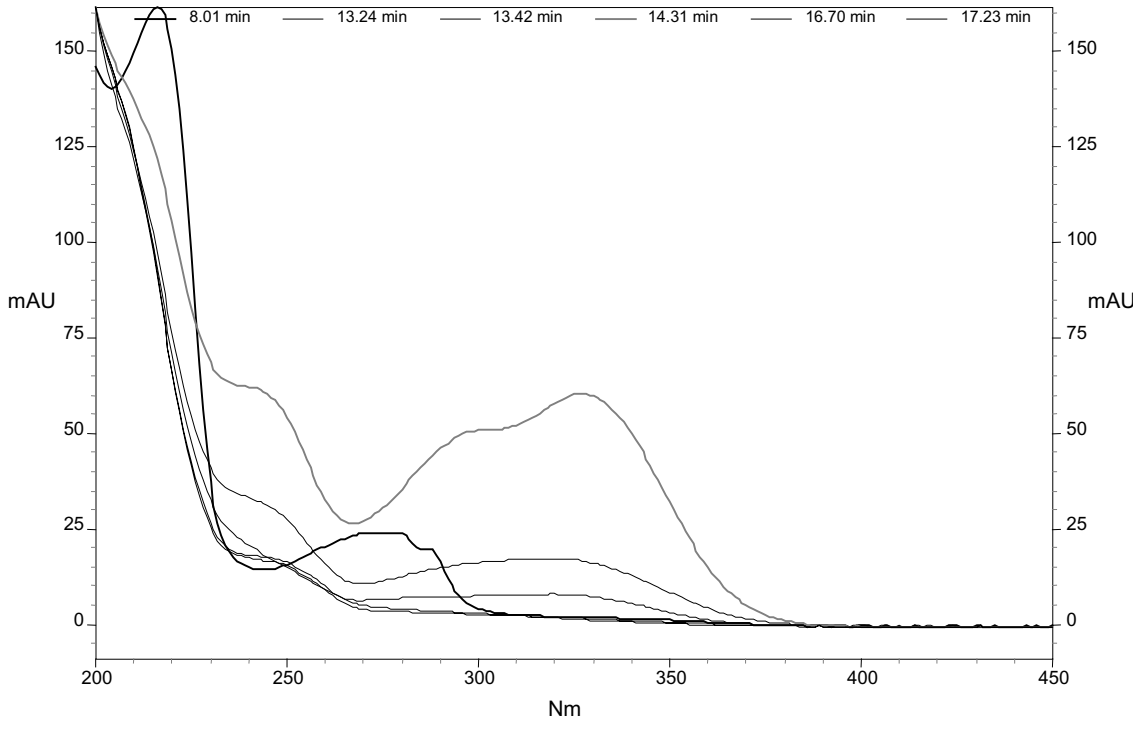

Figure.2. UV-Visible spectra of unknown peaks 1, 7, 8, 9, 10 and 11 from carrot tissues. Unknown peak 1: at 8.01 min (retention time); unknown peak 7: at 13.24 min; unknown peak 8: at 13.42 min; unknown peak 9: at 14.31min; unknown peak 10: at 16.70 min; unknown peak 11: 17.23 min.

in different tissues detected by the Folin-Ciocalteu method decreased in the same order as the phenolic contents detected by HPLC method. However, greater levels of total phenolics were found in carrots using the Folin-Ciocalteu assay as compared to HPLC quantification. Howard et al. [27] found the same difference between two methods used for analyses. This is likely due to the additional detection of minor phenolics or other reducing compounds remaining in the extracts.

Figure 3 also shows the proportion of the peel, phloem and xylem tissues in carrots. The peel accounted for 10.9-11.0% of

Table 1. Phenolic content in different tissues of carrots measured by HPLC analysis.

Peak number	Phenolic compounds[a]	Phenolic content (mg/100 g fresh weight)[b]					
		Chibagosun			Hitomigosun		
		peel	phloem	xylem	peel	phloem	xylem
1	Unknown	0.07	0.11	0.11	0.23	0.53	0.30
2	3'-CQ	0.09	0.11	0.01	0.02	0.06	0.03
3	CQ	15.04	1.83	0.19	7.39	3.29	0.44
4	Caffeic acid	0.11	0.07	0.01	0.01	0.01	ND
5	c5'-CQ	3.51	0.54	0.13	1.43	0.61	0.11
6	4'pCQ	1.12	0.06	0.02	0.03	0.02	0.02
7	unknown HCe	0.19	0.03	ND	0.25	0.10	0.02
8	unknown HCe	0.52	0.06	ND	0.35	0.10	0.07
9	unknown HCe	1.43	0.27	ND	1.74	0.68	0.14
10	unknown HCe	0.11	ND	ND	0.80	0.02	ND
11	unknown HCe	0.07	0.01	ND	0.76	0.03	ND
12	3'4'-DCQ	0.28	0.01	ND	0.67	0.06	ND
13	3'5'-DCQ	1.84	0.06	ND	3.62	0.41	0.02

a 3'-CQ: 3'-caffeoylquinic acid, 5'-CQ: cafferoylquinic acid (chlorogenic acid), c5'-CQ: cis-5'-caffeoylquinic acid, 4'pCQ: 4'p-coumaroylquinic acid, 3',4'-DCQ: 3',4'-dicaffeoylquinic acid, 3',5'-DCQ: 3',5'-dicaffeoylquinic acid, HCe: hydroxycinnamic esters unidentified. b. The concentration of phenolic compounds were expressed in mg/100 g fresh weight equivalent chlorogenic acid for hydroxycinnamic acids except caffeic acid. The concentration of unknown peak 1 was expressed as mg gallic acid equivalent/100 μg fresh weight. Data are the means of three duplicate experiments. ND means not detectable.

the carrot fresh weight, while the phloem and xylem gave 65.5-65.6% and 23.4-23.6% respectively. Although carrot peel accounted for only 11.0% of the amount of the carrot fresh weight, it could provide 54.1% of the amount of the phenolics in 100 g fresh weight of carrots. The phloem tissue provides 39.5% while the xylem tissue provides only 6.4%. Recently, carrot has been popular in fast food and prepared salads because of its fresh-like quality. However, carrot peel, a processing by-product, is treated as the wastes. We suggest that the higher level of phenolic compounds in the peel could be considered for value-added utilization in the processing industry.

Antioxidant activity of phenolic extracts from different tissues of carrots: Antioxidant activities of phenolic extracts from different carrot tissues are shown in Table 2. The extracts were tested at 10% concentration level of the original extracts. In the Chibagosun carrots, the peel extract had high antioxidant activity of 80.1%, much higher than the phloem and xylem extracts. In the Hitomigosun carrot, antioxidant activity in peel was 75.4%, also much higher than the phloem and xylem extracts. In the study of Kähkönen et al. [25] using similar β-carotene bleaching method, the carrot peel and flesh were found to have 52% and 10% at 5000 ppm dry weight base. The different results are probably not only due to the use of different extraction methods and cultivars used but also the amount of the extracts used in the measurements of antioxidant activity. Antioxidant activity of carrots was also evaluated in two other studies [12, 26]. However, as different methods and expression for antioxidant activity were used in these studies, it is difficult to compare the results with ours. Chlorogenic acid was found to be the most abundant phenolic acid in the plant extracts and also the most active antioxidant. A 1.2×10^{-5} M solution of chlorogenic acid inhibited over 80% of peroxide formation in a linoleic acid test system [14]. We found that 150 μg/ml of pure chlorogenic acid only had antioxidant activity of 15.8% in the β-carotene bleaching system. Therefore, we suggest that other hydroxycinnamic derivatives such as dicaffeoylquinic acids may exert some strong antioxidant activities along with chlorogenic acid.

Table 2. Antioxidant activities of the extracts from different carrot tissues.

Extracts or compounds	Antioxidant activity (%)
Chibagosun peel	80.1
Chibagosun phloem	9.6
Chibagosun xylem	3.2
Hitomigosun peel	75.4
Hitomigosun phloem	18.8
Hitomigosun xylem	7.5
Chlorogenic acid (150 μg/ml)	15.8

[a] All the extracts were tested at 10% level of the original extracts. Data are the means of three duplicate experiments.

Free radical scavenging activity of phenolic extracts from different tissues of carrots: All the extracts from carrot tissues were found to possess DPPH-scavenging activity (Table 3). In the Chibagosun carrots, free radical scavenging activity in peel was 75.8%, much higher than in phloem and xylem. In the Hitomigosun carrot, antioxidant activity in peel was 67.4%, also much higher than in phloem and xylem. The quality of the antioxidants in the extracts was determined by the IC50 values shown in Table 3. A low IC50 indicates strong antioxidant activity in a sample tested. The radical scavenging activities of vitamin C, chlorogenic acid and β-carotene were measured with the same method and the results were also shown in Table 3. The differences in IC50 between the extracts may be because

Table 3. Free radical scavenging activity of the extracts from different carrot tissues.

Extracts or compounds	Free radical scavenging activity (%)[a]	IC50 (mM)
Chibagosun peel	75.8	0.30
Chibagosun phloem	10.0	0.28
Chibagosun xylem	3.5	0.34
Hitomigosun peel	67.4	0.27
Hitomigosun phloem	17.0	0.33
Hitomigosun xylem	5.5	0.35
Chlorogenic acid (100μg/ml)	36.2	0.39
Vitamin C (50 μg/ml)	30.2	0.47
α-Carotene (300μg/ml)	28.2	0.99

[a] All the extracts were tested at 10% level of the original extracts. Data are the means of three duplicate experiments.

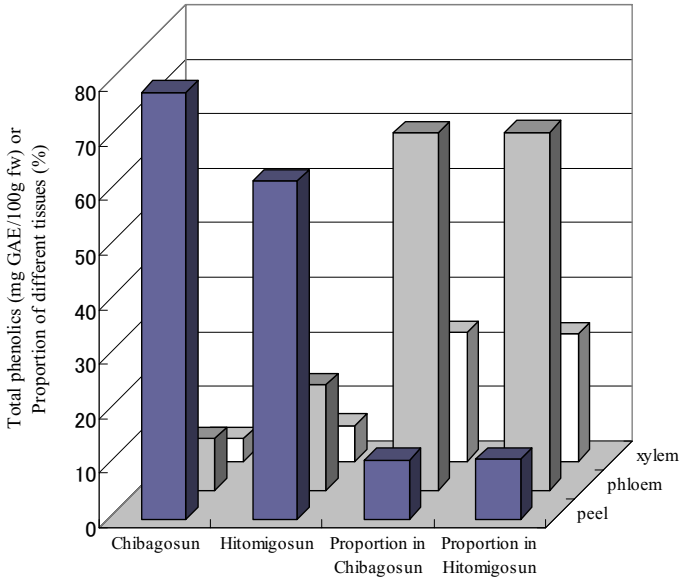

Figure.3. Distribution of total phenolics in peel, phloem and xylem tissues of carrots and proportion of the tissues. Data in total phenolics were the means of three duplicate experiments. Each data point in proportion of tissues represents the mean of 10 carrots.

the contents of individual phenolic compounds are different in the extracts and different phenolic compounds have different response to the test method. Lu and Foo [28] found that apple polyphenols had different IC50, indicating that they have different DPPH free radical scavenging capability. Wang and Jiao [29] found that chlorogenic acid was effective in inhibiting $OH^{\cdot}$ free radical activity at 13.9% and the scavenging capacity of chlorogenic acid on O_2^- and H_2O_2 was 2.43 and 8.14% respectively. In our study, all the extracts had IC50 values of 0.27-0.35 mM, indicating that their radical scavenging activities were higher than those in pure chlorogenic acid, vitamin C and β-carotene. These results were consistent with the findings reported by Lu and Foo [28]. Furthermore, procyanidin dimers (B1, B2 and B3) in apple and azuki beans were observed to be more effective in scavenging free radicals than monomer catechin and epicatechin [28, 30]. Therefore, we suggest that phenolics could play an important role in antioxidant properties in carrots, and other hydroxycinnamic derivatives such as dicaffeoylquinic acids may exert some strong radical scavenging activities along with chlorogenic acid.

Relationship between phenolic content and antioxidant properties in carrots: Many researches could not find significant correlation between the total phenolic content and antioxidant activity of the plant extracts. This may be because fruits and vegetables contain many different antioxidant components including carotenoids, vitamins, phenolic compounds, flavonoids, etc. [14] which could affect the measurement of antioxidant activity. α- and β-carotenes are abundant in carrots and the range of α- and β-carotenes was from 4.0 to 8.7 mg/100 g and from 6.9 to 16.1 mg/100 g [31]. Vitamin C is a potent reducing agent and acts as a free radical scavenger [32]. Its content varies between 2.8 and 5.0 mg/100 g in carrots [33]. Thus, carotenes and vitamin C in carrots may affect the measurement of antioxidant activity of phenolics. In our study, the Sep-Pak C18 cartridges were used to remove polar interfering compounds such as vitamin C prior to analysis. After Sep-Pak C18 cartridges eluted with methanol, the samples were converted to aqueous solutions by removal of methanol and then filtered through 0.45 μM Advantec filter. As the carotenes are not water soluble [34], they would also be removed from the extracts. Therefore, the effect of carotenes and vitamin C on the measurement of antioxidant activity should be limited in this study. Our results indicate that antioxidant activity and radical scavenging activity correlated well with total phenolic contents in carrots respectively (Figures 4 and 5). These results were consistent with the results reported by Lee et al. [35] in which a linear relationship between flavonoid concentration and antioxidant activity in peppers after using Sep-Pak C18 cartridges was observed.

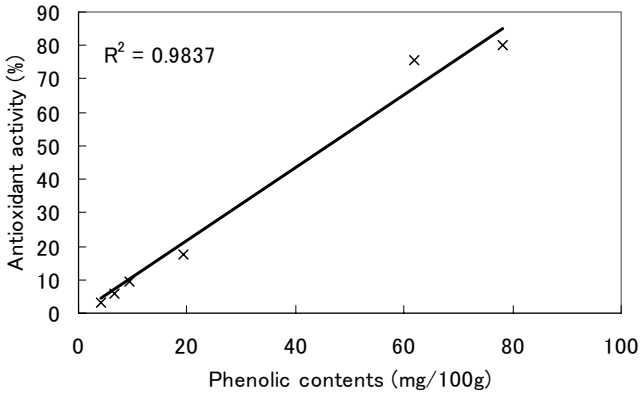

Figure.4. Relationship between antioxidant activity and total phenolic contents in carrots.

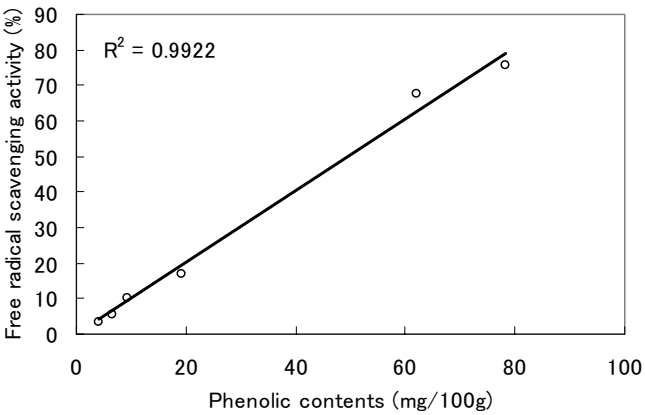

Figure.5. Relationship between free radical scavenging activity and total phenolic contents in carrots

Conclusion

Carrots contained mainly hydroxycinnamic acids and derivatives. Among them chlorogenic acid was a major hydroxycinnamic acid. Phenolic content in different tissues decreased from peel, phloem to xylem, while antioxidant and radical scavenging activities in different tissues decreased in same order as the phenolic content and correlated well with total phenolic contents. All phenolic extracts presented stronger radical scavenging ability than pure chlorogenic acid, vitamin C and β-carotene. Therefore, we suggest that phenolics could play an important role in antioxidant properties in carrots and

other hydroxycinnamic derivatives such as dicaffeoylquinic acids in the extracts may exert some strong antioxidant activities along with chlorogenic acid. The higher level of phenolics and antioxidant properties in carrot peel treated as the waste in the processing industry could be considered for value-added utilization.

Acknowledgement

We would like to thank Japan Society for Promotion of Sciences for financial assistance to this research.

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