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Effects of cultivation year and growing location on the phenolic profile of differently coloured carrot cultivars

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Summary

Carrots (*Daucus carota* L.) are economically and nutritionally important crops that, apart from carotenoids, contain numerous phenolic compounds which are assumed to exert health beneficial effects. The total phenolic contents of fruits and vegetables are known to depend on cultivar and growing conditions; however, studies examining the variability of a collection of carrots comprising differently coloured cultivars are rare. Therefore, the objective of the present study was to investigate the phenolic compounds of ten differently coloured carrot cultivars considering the effects of three cultivation years at two growing locations.

Although total phenolic contents varied in a wide range, both purple cultivars 'Anthonina' and 'Deep Purple' significantly exceeded those of yellow, orange, red, and uncoloured cultivars ($P \leq 0.05$) with amounts from 4,113 to 11,737 mg [kg dry matter (DM)]$^{-1}$. In contrast to the purple roots, the other generally were characterised by far lower polyphenol contents ranging from 33 to 1,369 mg (kg DM)$^{-1}$. Interestingly, the values did not considerably vary within these cultivars. In the present study, contrary to cultivar specific effects, the influence of growing location was found to be rather weak, supposedly due to similar climatic conditions at both locations. Similarly, variation of phenolic contents from year-to-year was less pronounced. In conclusion, the selection of breeding material was found to be of utmost importance regarding the expression of polyphenols in differently coloured carrots.

Introduction

Beneficial effects of phytochemicals on human health have been demonstrated in numerous studies. Phytochemicals are plant secondary metabolites, with phenolic compounds forming one of the most important groups. More than 10,000 phenolic compounds have so far been identified in a wide range of different plant materials. They can be classified into various sub-groups according to their number and arrangement of carbon atoms, comprising phenolic acids, flavonoids, stilbenes, and lignans (Carle, 2007; 2010). Phenolic acids can be further sub-divided into two groups based on their basic carbon skeleton, namely cinnamic acid derivatives and benzoic acid derivatives, while hydroxycinnamic acids are more common (Manach et al., 2004).

Due to the fact that phenolic compounds are widely distributed within fruits and vegetables, they constitute an important factor in plant-based human diets (Bravo, 1998; Kammerer et al., 2005). Their beneficial health effects are associated with the prevention of the risk of cardiovascular diseases, cancer, and cataract formation, as well as a number of other degenerative diseases (Shahidi and Naczk, 2004). Such health benefits are ascribed to their antioxidant activity, due to their chemical nature predestined for free radical scavenging (Leopoldini et al., 2011; Rice-Evans et al., 1997), as well as antimicrobial, antiviral, and further pharmacological actions (Shahidi and Naczk, 2004).

Carrots (*Daucus carota* L.) are a popular vegetable cultivated worldwide. Traditionally, in the northern hemisphere, it may be considered as the most important carotene source for human nutrition. However, as can be deduced from their browning potential, carrots also contain a variety of phenolic compounds, mainly phenolic acids that may be easily oxidised in the presence of oxygen. The most abundant compounds in carrot roots are 5-*O*-caffeoylquinic acid (chlorogenic acid), 3-*O*-caffeoylquinic acid (neochlorogenic acid), 3,4- and 3,5-di-*O*-caffeoylquinic acids, 3-, 4-, and 5-*O*-feruloylquinic acid, or 3- and 5-*p*-coumaroylquinic acids (Alasalvar et al., 2001; Klaiber et al., 2005). Consequently, phenolic acids in carrots have been associated with a protective effect against bacterial infection (Babic et al., 1993), interacting with proteins in their oxidised form (Friedman, 1997), and also influencing sensory properties (Phan, 1974). According to previous reports, bitter and sour taste perceptions were intensified with increasing total soluble phenolics (Talcott and Howard, 1999a), and a di-caffeic acid derivative showed a clear-cut correlation with bitterness in raw carrots (Kreutzmann et al., 2008). Furthermore, phenolic acids were shown to be directly involved in colour degradation (Talcott and Howard, 1999b) and enzymatic browning (Chubey and Nylund, 1969) of carrots.

The total phenolic contents of differently coloured carrot roots ranged from 7.7 to 74.6 mg (100 g fresh weight)$^{-1}$ (Alasalvar et al., 2001), and the wide range of values was shown to be cultivar dependent (Bozalan and Karadeniz, 2011; Metzger and Barnes, 2009; Nicolle et al., 2004; Sun et al., 2009; Talcott and Howard, 1999a). In addition, polyphenol levels may be affected by numerous other factors, including growth conditions, such as climate (Manach et al., 2004), location (Talcott and Howard, 1999a), and fertilisation (Smolén and Sady, 2009), as well as post-harvest conditions including storage (Heredia and Cisneros-Zevallos, 2009; Klaiber et al., 2005; Kramer et al., 2012; Sarkar and Phan, 1979) and processing (Gonçalves et al., 2010; Howard et al., 1994).

Theoretically, differing growing locations and cultivation years entail different environmental influences, which may lead to varying phenolic profiles and levels in various coloured carrot cultivars. Therefore, the present study was conducted to provide evidence about the effect of cultivar and cultivation on the content and profile of phenolic compounds in methanolic extracts of ten differently coloured carrot cultivars (white, yellow, orange, red, and purple roots) as investigated by HPLC analyses. The effects of two different European locations (Poland and Germany) over three consecutive growing seasons in 2008, 2009, and 2010 were also considered.

Materials and methods

Plant material

The roots of ten carrot cultivars (*Daucus carota* L.) of different origins and colours from field trial studies undertaken in 2008, 2009, and 2010 were included in the analysis. 'White Satin'

(WS), 'Yellowstone' (YS), 'Nerac' (NF), and 'Deep Purple' (DP) were obtained from Bejo Samen (Sonsbeck, Germany), and 'Line 710015' (LI), 'Nutrired' (NR), 'Santa Cruz' (SC), and 'Anthonina' (AN) from Seminis Vegetable Seeds (Neustadt, Germany). 'Blanche ½ longue des Vosges' (BV) was a Julius Kühn-Institute selection from gene bank accession 126 of the Institut National d'Horticulture (Angers, France), and 'Pusa Kesar' (PK) was from Warwick Genetic Resources Unit, Warwick University (Wellesbourne, UK).

The cultivars were grown in a collaborative research project at two different geographical locations near Kraków, Poland (Malopolska Region; 50° 07' N, 19° 59' E) [PL] on loess-brown soil and in Quedlinburg, Germany (North Harz Foreland Region; 51° 47′ N, 11° 8′ E) [D] on loess-black soil in 2008, 2009, and 2010 with four replications in each case. Field trial characteristics at the two locations are specified in Tab. 1.

After harvest aliquots of approx. 1 kg of carrot roots (marketable quality) of each cultivar and each replication were collected. The samples were washed with tap water, drained, sliced into cubes (1x1x1 cm), and frozen at -80 °C. In 2008, the samples were lyophilised at Hohenheim University. In 2009 and 2010, lyophilisation was carried out by GFT Sitte (Oppin, Germany). Finally, the carrot samples were ground in a centrifugal mill ZM1 (Retsch GmbH, Haan, Germany) in 2008 and with a ball mill MM 301 (Retsch GmbH, Haan, Germany) in 2009 and 2010. Subsequently, the powder was stored at -20 °C until analysis.

Phenolic compound analysis

Extraction and fractionation of phenolic compounds

Phenolic compounds were isolated according to the procedure described by KAMMERER et al. (2004) with minor modifications. In brief, aliquots of 1 g carrot powder were extracted by stirring with 20 ml of methanol/water (60/40, *v/v*, acidified with 0.01% HCl) for 1 h after flushing the flask with nitrogen. The extracts were centrifuged (10 min, 3480*g*), and the material was re-extracted with 20 ml of the same solvent (30 min). After filtration the combined supernatants were evaporated to dryness *in vacuo* at 30 °C, and the residue was dissolved in 3 ml of deionised water (pH 3.5).

The resulting extract was used for further purification. Fractionation of phenolic compounds was performed using end-capped C_{18}-Sep-Pak cartridges (Chromabond 1000, Macherey-Nagel, Düren, Germany) which were activated with 3 ml of methanol and rinsed with 10 ml of deionised water.

An aliquot of 2.5 ml of the extract was made up to 5 ml with deionised water. After adjusting the pH to 7.0, the solutions were applied to the cartridge. Non-anthocyanin phenolics were subsequently eluted with 10 ml of deionised water and 10 ml of 0.01% HCl (fraction I) and 20 ml of ethyl acetate (fraction II). Fraction III containing anthocyanins, which was eluted with methanol/0.01% HCl, was discarded. The eluates were concentrated *in vacuo*, and the residues obtained were dissolved in 50% aqueous methanol (fraction I) and in pure methanol (fraction II), respectively, membrane-filtered (0.45 µm), and used for HPLC-DAD-MS^n analyses. All analyses were performed in duplicate.

Chromatographic separation of phenolic compounds coupled with diode array and MS detection

Chromatographic separation was performed on a Phenomenex (Torrance, CA, USA) C_{18} Hydro-Synergi column (150 x 3.0 mm i.d., 4 µm particle size) equipped with a C_{18} ODS guard column (4.0 x 2.0 mm i.d.). Column temperature was 25 °C. The mobile phase consisted of 2% (*v/v*) acetic acid in water (eluent A) and 0.5% acetic acid in water and methanol (10/90, *v/v*; eluent B). The gradient used for the separation of phenolics in fraction I was 0% B to 35% B (35 min), 35% B to 75% B (20 min), 75% B to 100% B (2 min),

Tab. 1: Field trial characteristics of the two growing locations in the years 2008, 2009, and 2010

Location		Kraków [PL]	Quedlinburg [D]
2008	Vegetation period	16.05.2008-23.09.2008	14.05.2008-17.09.2008
	Temperature [°C]	16.9	17.0
	Soil temperature [°C]	18.2	19.0
	Rel. humidity [%]	n/a	79.3
	Rainfall [mm]	317.9	226.4
	Irrigation [mm]	+15 (2 applications)	+21 (3 applications)
	Total water [mm]	332.9 or 2.6/day	247.4 or 2.0/day
2009	Vegetation period	02.07.2009-08.10.2009	13.05.2009-08.09.2009
	Temperature [°C]	17.4	16.9
	Soil temperature [°C]	17.3	17.5
	Rel. humidity [%]	n/a	80.3
	Rainfall [mm]	178.3	284.8
	Irrigation [mm]	+0	+20 (2 applications)
	Total water [mm]	178.3 or 1.8/day	304.8 or 2.6/day
2010	Vegetation period	08.06.2010-28.09.2010	18.05.2010-13.09.2010
	Temperature [°C]	17.6	16.9
	Soil temperature [°C]	19.2	18.0
	Rel. humidity [%]	n/a	85.0
	Rainfall [mm]	451.2	274.2
	Irrigation [mm]	+0	+20 (2 applications)
	Total water [mm]	451.2 or 4.0/day	294.2 or 2.5/day

n/a not available

100% B isocratic (5 min), 100% B to 0% B (2 min). For fraction II a gradient programme was used as follows: 0% B to 100% B (83 min), 100% B isocratic (5 min), 100% B to 0% B (2 min). Total run time was 69 min and 95 min, respectively. The injection volume for all samples ranged from 5-40 µl. Polyphenol separation was monitored separately at 280 nm (hydroxybenzoic acids) and 320 nm (hydroxycinnamic acids) at a flow rate of 0.4 ml/min. Additionally, UV/Vis spectra were recorded in the range of 200-600 nm at a spectral acquisition range of 1.25 scans/s (peak width 0.2 min).

Analyses were performed using a series 1100 HPLC system (Agilent, Waldbronn, Germany), equipped with a degasser, a binary gradient pump, a thermoautosampler, a column oven, and a diode array detection (DAD) system controlled by Agilent ChemStation software (ver. A.09.03). The system was coupled on-line with a Bruker (Bremen, Germany) Esquire 3000+ ion trap mass spectrometer fitted with an ESI source. Data acquisition and processing were performed using Esquire Control software (ver. 3.1). Negative ion mass spectra of the column effluent were recorded in the range *m/z* 50-1000 at a scan speed of 13,000 Th/s (peak width 0.6 Th, FWHM). Nitrogen was used both as drying gas at a flow rate of 9.0 l/min and as nebulising gas at a pressure of 40.0 psi. The nebuliser temperature was set at 365 °C. Helium was used as collision gas for collision-induced dissociation (CID). The fragmentation amplitude was 1.5 V.

Characterisation and quantitation of phenolic compounds

Phenolic compounds and one amino acid were quantitated using calibration curves of the respective standards or related reference compounds [caffeic acid, quercetin dihydrate, *p*-coumaric acid, *p*-hydroxybenzoic acid, and ferulic acid from Roth (Karlsruhe, Germany); chlorogenic acid, vanillic acid, and tryptophan from Sigma (St. Louis, MI, USA)] and molecular weight correction factors according to CHANDRA et al. (2001). The results were expressed in mg per kg dry matter (DM), as means of four replications. Characterisation of individual compounds was based on UV spectra, mass spectra, and retention times.

Statistical analysis

Data were subjected to analysis of variance (ANOVA). As a first step, means were separated by using Tukey's multiple comparison test ($P \leq 0.05$). Further, multivariate data analysis was performed, adopting a stepwise approach as described by KIENZLE et al. (2011) for harvest maturity specification of mango fruit with some modifications. The detected compounds of each variety (combination of cultivar from each growing location and cultivation year) were processed by means of principal component analysis (PCA) to find out similarities and differences between the varieties. For a closer distinction of the varieties, agglomerative hierarchical cluster analysis (CA) was further applied to the data matrices outlined above. The complete-linkage clustering was based on maximum Euclidian distances, considering all PCs. Dendrograms showed maximum distances as heights of the clusters. Data evaluation was performed with the SAS software package (SAS Institute, Cary, North Carolina; Software version 9.1).

Results

Characterisation of phenolic compounds and tryptophan

Tentative assignment of phenolic compounds and tryptophan in root extracts of differently coloured carrot cultivars was based on their chromatographic behaviour. Characteristic UV spectra, HPLC retention times, and HPLC-MS^n data matched those of authentic reference compounds and were in accordance with literature data (CLIFFORD, 2003; FANG et al., 2002; HOSSAIN et al., 2010; KAMMERER et al., 2004; KLAIBER et al., 2005). Whenever UV and LC-MS data of the compounds did not allow their unambiguous identification, the substances were designated as derivatives.

Basically, these investigations provided evidence of similar phenolic profiles of individual cultivars independent of different cultivation years and growing locations. Hence, Fig. 1 displays typical chromatographic profiles of fraction I of five carrot cultivars of different root colours (white, yellow, orange, red, and purple) cultivated in PL in 2008. Phenolic acids comprising a benzoic acid basic structure were detected at 280 nm because of their maximum absorption in a wavelength range of 200 - 290 nm (ROBBINS, 2003). Due to the additional conjugation, cinnamate derivatives showing a broad secondary absorbance band from 270 - 360 nm (ROBBINS, 2003) were monitored at 320 nm. HPLC and MS data of individual compounds are summarised in Tab. 2.

For the majority of compounds, base line separation was achieved. In general, 20 phenolic substances were identified, mainly hydroxycinnamic acids and hydroxybenzoic acids, one flavonoid, and one aromatic amino acid. The hydroxycinnamic acids comprised derivatives of caffeic acid, such as 3-*O*-caffeoylquinic acid, 5-*O*-*trans*-caffeoylquinic acid, 5-*O*-*cis*-caffeoylquinic acid, further monocaffeoylquinic acids, 4,5-di-*O*-caffeoylquinic acid, additional caffeic acid and di-caffeic acid derivatives, as well as ferulic acid derivatives (1-*O*-feruloylquinic acid, 4-*O*-feruloylquinic acid, 5-*O*-feruloylquinic acid, other feruloylquinic acids, and ferulic acid derivatives). Furthermore, a range of *p*-coumaric acid derivatives (5-*p*-coumaroylquinic acid) and of mixed conjugates of ferulic and caffeic acids (4-*O*-feruloyl-5-*O*-caffeoylquinic acid, caffeic / ferulic acid derivatives) were detected. Usually such compounds form simple esters with quinic acids or sugars. In contrast, ferulic acid was found in un-esterified form. The hydroxybenzoic acids were deduced from vanillic acid and *p*-hydroxybenzoic acid. Additionally, quercetin-3-*O*-galactoside and the aromatic amino acid tryptophan were detected.

Influence of cultivar on the contents of phenolic compounds

Content levels of the phenolic compounds and tryptophan were determined in ten carrot cultivars harvested in 2008, 2009, and 2010 on two growing locations (D and PL). In Tab. 3 mean contents of different carrot cultivars are presented, listed by carrot root colour and also including statistical significances ($P \leq 0.05$).

The highest amounts of total phenolics have been determined in purple carrots with values ranging from 4,113 to 11,737 mg (kg $DM)^{-1}$. In contrast, all other cultivars being devoid of anthocyanins showed considerably lower contents of colourless phenolics [33 to 1,369 mg (kg $DM)^{-1}$] (Tab. 3). Interestingly, despite this broad range, the total phenolic contents did not differ significantly within white, yellow, orange, and red carrot cultivars. Furthermore, mainly hydroxycinnamic acids were detected among phenolic acids in carrot roots (Tab. 2).

Considering the major phenolic compounds, a vanillic acid derivative dominated in 'White Satin', whereas ferulic acid derivatives prevailed in the yellow cultivar 'Yellowstone'. In cultivars 'Blanche ½ longue des Vosges' (white) and 'Line 710015' (yellow) the major compounds varied depending on cultivation year and growing location. In orange, red, and purple roots, predominantly 5-*O*-*trans*-caffeoylquinic acid (chlorogenic acid) was observed, yet the highest contents were found in purple cultivars 'Anthonina' and 'Deep Purple' where it constantly represented more than 50% of the total phenolic content. Although there was a great range of chlorogenic acid contents in white, yellow, orange, and red roots (0 - 64% of total phenolic contents), these amounts did not significantly differ. Moreover, 5-*O*-*cis*-caffeoylquinic acid was detected in all cultivars, showing highest quantities in purple roots. In contrast to chloro-

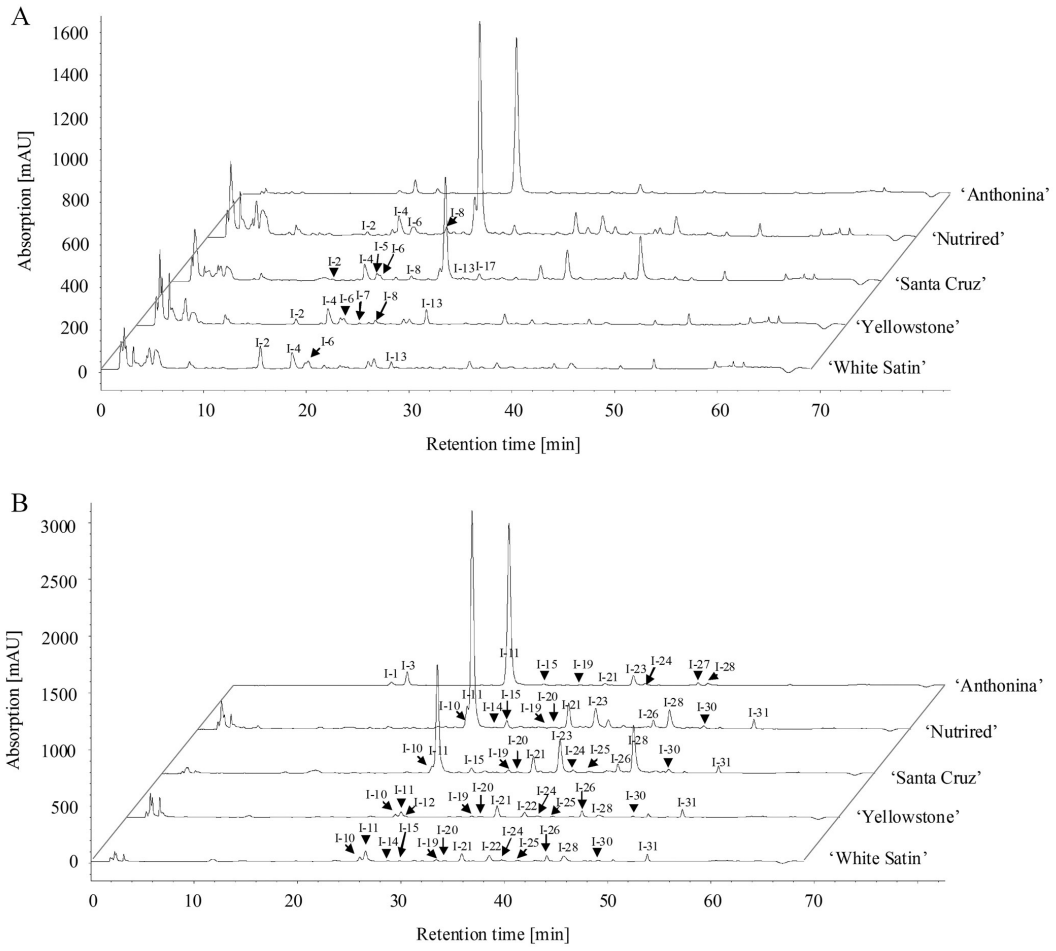

Fig. 1: Chromatographic profiles at a wavelength of (A) 280 nm and (B) 320 nm of fraction I of five differently coloured carrot cultivars (samples from 2008, PL). For peak assignment see Tab. 2.

genic acid, trace amounts of 3-*O*-caffeoylquinic acid (neochlorogenic acid) were determined only in 'Pusa Kesar' (red), while higher amounts were found in both purple cultivars 'Anthonina' and 'Deep Purple'. Further caffeic acid derivatives were only present in minor amounts in white and yellow cultivars. However, in orange, red, and purple roots these compounds have been found in increased amounts. While ferulic acid derivatives dominated with 28%, 49%, 55%, and 48% in the roots of 'White Satin', 'Blanche ½ longue des Vosges', 'Yellowstone', and 'Nutrired', respectively, they occurred in far lower contents in all other cultivars. Free ferulic acid was detected in six ('White Satin', 'Blanche ½ longue des Vosges', 'Yellowstone', 'Santa Cruz', 'Anthonina' and 'Deep Purple') of ten cultivars. Derivatives of caffeic and ferulic acids were primarily detected in purple cultivars. All other cultivars showed far lower amounts, except for 'Line 710015'. The phenolic acid 5-*p*-coumaroylquinic acid constituted a minor constituent of all cultivars under investigation.

Among hydroxybenzoic acids, vanillic acid derivatives, and a *p*-hydroxybenzoic acid hexoside were quantitated. Interestingly, *p*-hydroxybenzoic acid hexoside was not found in white and yellow cultivars. Remarkably, purple carrots were devoid of hydroxybenzoic acids. Instead, the flavonoid quercetin-3-*O*-galactoside was detected, which did not occur in the remaining cultivars. Finally, tryptophan was determined in all carrot roots, most frequently in red cultivars.

Influence of cultivation year on phenolic contents

Due to the relatively large number of cultivars and compounds detected, multivariate data analyses were performed. Hence, the mean values of the data set were first sorted by growing location. Generally, on both locations (D and PL) purple cultivars could be clearly discriminated from the differently coloured carrot roots (Fig. 2). Among purple carrots 'Anthonina' and 'Deep Purple', the results of year 2008 were outstanding, whereas the values of 2009 and 2010 did not differ considerably. Surprisingly, also the score for 'Pusa Kesar' of year 2008 took a separate position (Fig. 2). While in D this variety was likely to be an outlier, the content of roots grown in PL was merged in one cluster comprising non-purple coloured cultivars. The values of all other roots were grouped in approximate positions.

Considering their total phenolic contents, individual cultivars behaved differently depending on cultivation years. No clear trend could be detected. Throughout the years the most striking differences in contents were found for roots of 'Pusa Kesar' the amounts of which dropped from 2008 to 2010 by a factor of 8.5 and 4.6-fold in PL and D, respectively.

Influence of growing location on phenolic contents

Regarding the effect of location, PCA and CA were performed with means sorted by cultivation year. In agreement with the observations described above, purple cultivars 'Anthonina' and 'Deep Purple' stood out compared to the cultivars being devoid of anthocyanins (Fig. 3). However, only in 2010 purple roots of both growing locations were found in separate positions. In 2008 and 2009, the scores for purple cultivar 'Anthonina' were clearly separated from

Tab. 2: Characteristic data of phenolic compounds from different carrot cultivars. Peak numbers and retention times refer to HPLC traces in Fig. 1.

Peak no.	Retention time [min]	HPLC/DAD UV spectrum λ_{max} [nm]	$[M-H]^-$ *m/z*	HPLC/ESI(-)-MS^n experiment *m/z* (% base peak)	Tentative identification
Fraction I					
I-1	14.9	238, 308sh, 327	365	MS^2[365]: 203(100) MS^3[365→203]: 97(100), 129(52)	Caffeic acid derivative
I-2	16.0	227, 254, 292	659	MS^2[659]: 329(100) MS^3[659→329]: 167(100)	Vanillic acid derivative
I-3	16.9	235, 308sh, 325	353	MS^2[353]: 191(100), 179(86)	3-*O*-Caffeoylquinic acid[a]
I-4	19.1	229, 278	203	MS^2[203]: 116(100)	Tryptophan[a]
I-5	20.1	255	299	MS^2[299]: 137(100) MS^3[299→137]: 93(100)	*p*-Hydroxybenzoic acid hexoside
I-6	20.6	283	472	MS^2[472]: 404(100), 300(66) MS^3[472→404]: 275(100), 386(67)	Not identified
I-7	23.7	258	329	MS^2[329]: 125(100) MS^3[329→125]: 97(100)	Not identified
I-8	23.8	268	443	MS^2[443]: 161(100), 237(79), 437(71), 281(58) MS^3[443→161]: 101(100)	Not identified
I-9	25.2	298	508	MS^2[508]: 405(100) MS^3[508→405]: 276(100)	Not identified
I-10	26.8	234, 291, 314	355	MS^2[355]: 193(100) MS^3[355→193]: 149(100), 134(73)	Ferulic acid derivative
I-11	27.3	240, 307sh, 326	353	MS^2[353]: 191(100)	5-*O*-*trans*-Caffeoylquinic acid[a] (chlorogenic acid)
I-12	28.2	252, 327	658	MS^2[658]: 385(100) MS^3[658→385]: 191(100), 193(58)	Not identified
I-13	28.5	258	431	MS^2[431]: 329(100) MS^3[431→329]: 125(100), 203(58)	Not identified
I-14	29.5	294, 318	355	MS^2[355]: 193(100) MS^3[355→193]: 134(100), 149(87)	Ferulic acid derivative
I-15	30.3	233, 316	353	MS^2[353]: 191(100)	5-*O*-*cis*-Caffeoylquinic acid
I-16	31.0	250, 327	365	MS^2[365]: 185(100), 203(94) MS^3[365→185]: 141(100)	Caffeic acid derivative
I-17	32.5	269	281	MS^2[281]: 237(100) MS^3[281→237]: 171(100), 123(89), 207(74)	Not identified
I-18	32.8	312	658	MS^2[658]: 385(100) MS^3[658→385]: 191(100), 193(86)	Not identified
I-19	33.9	232, 312	337	MS^2[337]: 191(100)	5-*p*-Coumaroylquinic acid[a]
I-20	34.3	239, 308	367	MS^2[367]: 173(100)	4-*O*-Feruloylquinic acid
I-21	36.5	237, 305sh, 326	367	MS^2[367]: 191(100)	5-*O*-Feruloylquinic acid
I-22	39.1	239, 308sh, 328	367	MS^2[367]: 191(100)	1-*O*-Feruloylquinic acid
I-23	39.8	241, 308sh, 329	365	MS^2[365]: 203(100) MS^3[365→203]: 115(100), 97(90), 85(76), 69(50)	Caffeic acid derivative
I-24	39.9	238, 303sh, 323	193	MS^2[193]: 134(100)	Ferulic acid[a]
I-25	41.6	235, 324	379	MS^2[379]: 341(100), 185(69), 203(64) MS^3[379→341]: 179(100)	Caffeic acid derivative
I-26	44.6	237, 308sh, 328	379	MS^2[379]: 185(100) MS^3[379→185]: 141(100)	Ferulic acid derivative
I-27	44.9	243, 307sh, 326	193	MS^2[193]: 131(100), 134(79)	Ferulic acid derivative, *e.g.* isoferulic acid
I-28	45.6	238, 308sh, 328	527	MS^2[527]: 365(100) MS^3[527→365]: 203(100)	di-Caffeic acid derivative
I-29	47.7	238, 308sh, 326	541	MS^2[541]: 379(100) MS^3[541→379]: 185(100)	Caffeic / ferulic acid derivative
I-30	48.9	234, 308sh, 327	541	MS^2[541]: 379(100) MS^3[541→379]: 185(100)	Caffeic / ferulic acid derivative
I-31	54.2	229, 304sh, 324	577	MS^2[577]: 355(100), 193(61), 505(53) MS^3[577→355]: 355(100), 163(70)	Ferulic acid derivative
Fraction II					
II-1	17.2	267, 296	329	MS^2[329]: 167(100), 209(72)	Vanillic acid hexoside
II-2	17.7	230, 279	203	MS^2[203]: 159(100)	Tryptophan[a]
II-3	17.9	230, 279	431	MS^2[431]: 329(100), 125(88) MS^3[431→329]: 203(100), 125(56)	Not identified

Peak no.	Retention time [min]	HPLC/DAD UV spectrum λ_{max} [nm]	$[M-H]^-$ *m/z*	HPLC/ESI(-)-MS^n experiment *m/z* (% base peak)	Tentative identification
II-4	19.3	239, 306sh, 326	353	MS^2[353]: 173(100), 179(56)	Caffeoylquinic acid
II-5	20.1	239, 306sh, 326	341	MS^2[341]: 281(100), 179(94), 251(53) MS^3[341→281]: 179(100)	Caffeic acid derivative
II-6	20.2	232, 304sh, 325	355	MS^2[355]: 217(100) MS^3[355→217]: 202(100)	Not identified
II-7	21.0	240, 307sh, 326	353	MS^2[353]: 173(100), 191(81)	Caffeoylquinic acid
II-8	22.3	235, 304sh, 327	341	MS^2[341]: 281(100), 179(65) MS^3[341→281]: 179(100)	Caffeic acid derivative
II-9	23.1	235, 304sh, 325	355	MS^2[355]: 217(100) MS^3[355→217]: 202(100)	Not identified
II-10	23.8	238, 306sh, 327	353	MS^2[353]: 173(100), 191(86)	Caffeoylquinic acid
II-11	24.7	242, 307sh, 327	353	MS^2[353]: 191(100)	5-*O*-*trans*-Caffeoylquinic acid[a] (chlorogenic acid)
II-12	25.5	242, 304sh, 330	355	MS^2[355]: 193(100), 217(58) MS^3[355→193]: 134(100)	Ferulic acid derivative
II-13	25.8	258	431	MS^2[431]: 329(100) MS^3[431→329]: 125(100), 203(75), 179(57)	Not identified
II-14	26.4	237, 328	355	MS^2[355]: 193(100), 217(64) MS^3[355→193]: 134(100), 149(49)	Ferulic acid derivative
II-15	27.8	240, 305sh, 324	355	MS^2[355]: 265(100), 235(97), 295(78), 193(65) MS^3[355→265]: 193(100)	Ferulic acid derivative
II-16	27.1	240, 316	353	MS^2[353]: 191(100)	5-*O*-*cis*-Caffeoylquinic acid
II-17	30.4	236, 306sh, 326	355	MS^2[355]: 265(100), 295(90), 235(77) MS^3[355→265]: 193(100)	Ferulic acid derivative
II-18	31.0	306sh, 328	367	MS^2[367]: 191(100)	Feruloylquinic acid
II-19	32.2	208, 306sh, 326	367	MS^2[367]: 191(100)	Feruloylquinic acid
II-20	34.1	238, 305sh, 326	367	MS^2[367]: 191(100)	Feruloylquinic acid
II-21	35.6	237, 303sh, 323	193	MS^2[193]: 134(100)	Ferulic acid[a]
II-22	37.4	236, 307sh, 326	365	MS^2[365]: 203(100) MS^3[365→203]: 97(100)	Caffeic acid derivative
II-23	38.7	230, 268, 294sh	373	MS^2[373]: 193(100), 343(76), 219(59) MS^3[373→193]: 178(100)	Not identified
II-24	39.1	231, 278	565	MS^2[565]: 361(100) MS^3[565→361]: 165(100), 361(91), 346(69), 179(58)	Not identified
II-25	41.4	239, 308sh, 327	527	MS^2[527]: 365(100) MS^3[527→365]: 203(100)	di-Caffeic acid derivative
II-26	42.8	256, 356	463	MS^2[463]: 301(100) MS^3[463→301]: 301(100)	Quercetin-3-*O*-galactoside
II-27	44.0	243, 306sh, 329	515	MS^2[515]: 353(100) MS^3[515→353]: 173(100)	4,5-di-*O*-Caffeoylquinic acid
II-28	46.4	237, 306sh, 323	541	MS^2[541]: 379(100) MS^3[541→379]: 185(100)	Caffeic / ferulic acid derivative
II-29	47.9	236, 308sh, 328	529	MS^2[529]: 367(100) MS^3[529→367]: 173(100), 193(53)	4-*O*-Feruloyl-5-*O*-caffeoylquinic acid
II-30	50.9	240, 308sh, 328	531	MS^2[531]: 337(100) MS^3[531→337]: 217(100), 193(98), 337(92), 175(91)	Ferulic acid derivative
II-31	52.3	235, 323	503	MS^2[503]: 267(100), 311(73) MS^3[503→267]: 267(100), 252(71)	Not identified
II-32	54.9	235, 303sh, 325	577	MS^2[577]: 355(100), 193(69) MS^3[577→355]: 163(100), 355(64)	Ferulic acid derivative
II-33	58.9	265	327	MS^2[327]: 229(100), 211(70) MS^3[327→229]: 211(100), 229(51)	Not identified
II-34	62.8	256	329	MS^2[329]: 229(100), 211(65) MS^3[329→229]: 229(100), 230(97), 127(58), 211(58)	Not identified
II-35	64.5	235, 307	385	MS^2[385]: 279(100), 383(95), 367(93), 337(66), 342(59) MS^3[385→279]: 279(100)	Not identified
II-36	65.2	261	615	MS^2[615]: 495(100) MS^3[615→495]: 137(100)	Not identified
II-37	71.2	228, 269	597	MS^2[597]: 459(100) MS^3[597→459]: 415(100)	Not identified

[a] identification with standard

Tab. 3: Mean contents (n=4) of individual phenolic compounds in differently coloured carrot roots.

	Phenolic content [mg (kg DM)$^{-1}$]											
	D			PL			D			PL		
	2008	2009	2010	2008	2009	2010	2008	2009	2010	2008	2009	2010
White cultivars	'White Satin'						'Blanche ½ longue des Vosges'					
Vanillic acid derivative	118.3 b B	74.8 a A	87.2 a B	97.1 b A	92.2 b A	64.3 a A	33.9 a A	38.3 a B	33.5 a B	32.6 b A	32.6 b A	20.6 a A
Vanillic acid hexoside	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
Tryptophan	41.4 a A	nd a	nd a	43.7 b A	nd a	nd a	11.4 a A	nd a	nd a	19.7 b A	nd a	nd a
p-Hydroxybenzoic acid hexoside	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
3-*O*-Caffeoylquinic acid	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
5-*O*-*trans*-Caffeoylquinic acid	1.9 a A	0.8 a A	0.4 a A	21.9 a B	29.3 a B	37.7 a B	2.8 a A	nd a A	10.2 a A	70.3 b B	4.0 a B	37.6 ab B
5-*O*-*cis*-Caffeoylquinic acid	nd a	nd a A	0.1 b A	nd a	8.3 c B	2.9 b B	nd a A	nd a A	0.8 a A	2.4 ab B	0.2 a A	4.1 b B
Caffeoylquinic acid	9.4 c A	4.7 b A	0.0 a A	35.1 c B	20.3 b B	5.3 a B	nd	nd	nd	nd	nd	nd
4,5-di-*O*-Caffeoylquinic acid	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
Caffeic acid derivative	3.3 b A	nd a	nd a	1.8 b A	nd a	nd a	0.8 b A	nd a A	nd a	32.3 b B	2.8 a A	nd a
di-Caffeic acid derivative	1.1 a A	nd a	nd a	15.3 b A	nd a	nd a	0.2 a A	nd a	nd a	34.9 b B	nd a	nd a
5-*p*-Coumaroylquinic acid	1.3 a A	nd a	nd a A	1.7 b A	nd a	0.6 a B	2.7 a A	nd a	1.4 a A	4.1 a A	nd a	0.7 a B
Ferulic acid	2.2 b A	nd a	nd a	1.4 b A	nd a	nd a	1.2 b A	nd a	nd a	2.6 b B	nd a	nd a
1-*O*-Feruloylquinic acid	4.4 b A	1.0 a B	nd a A	13.0 b B	nd a A	1.0 a B	2.2 b A	nd a A	0.9 ab A	23.3 b B	1.4 a A	1.3 a A
4-*O*-Feruloylquinic acid	nd A	nd	nd	1.4 b B	nd a	nd a	0.4 a A	nd a	nd a	2.7 b B	nd a	nd a
5-*O*-Feruloylquinic acid	10.0 b A	1.1 a A	0.4 a A	14.4 b A	nd a A	2.0 a B	7.2 b A	nd a A	2.4 ab A	26.3 b B	3.7 a A	2.6 a A
Feruloylquinic acid	nd a A	19.8 b A	3.2 a A	5.8 a B	62.6 b B	9.3 a B	11.4 a A	62.9 b A	33.4 a A	19.0 a B	97.5 b B	20.8 a A
Ferulic acid derivative	54.8 b A	23.0 a A	13.0 a A	40.2 c A	24.6 b A	10.8 a A	17.6 b A	18.3 b A	10.1 a B	38.2 c B	13.5 b A	5.2 a A
4-*O*-Feruloyl-5-*O*-caffeoylquinic acid	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
Caffeic / ferulic acid derivative	nd A	nd	nd	1.6 b B	nd a	nd a	nd A	nd	nd	5.5 b B	nd a	nd a
Quercetin-3-*O*-galactoside	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
Total phenolics	**248.1 b A**	**125.2 a A**	**104.3 a A**	**294.4 b A**	**237.4 b B**	**133.9 a B**	**91.7 a A**	**119.4 a A**	**92.7 a A**	**313.8 b B**	**155.7 a A**	**92.9 a A**
Yellow cultivars	'Yellowstone'						'Line 710015'					
Vanillic acid derivative	32.8 b A	24.8 ab A	20.4 a B	23.1 ab A	27.4 b A	15.2 a A	16.7 a A	11.5 a A	52.8 b B	11.7 a A	10.2 a A	9.8 a A
Vanillic acid hexoside	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
Tryptophan	20.7 b A	nd a	nd a	25.8 b A	nd a	nd a	14.4 b A	nd a A	nd a	16.0 b A	4.6 a B	nd a
p-Hydroxybenzoic acid hexoside	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
3-*O*-Caffeoylquinic acid	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
5-*O*-*trans*-Caffeoylquinic acid	nd a A	0.3 a A	15.8 b A	19.7 a B	5.6 a B	21.1 a A	0.4 a A	4.5 a A	9.8 a A	7.7 a B	4.9 a A	85.0 b B
5-*O*-*cis*-Caffeoylquinic acid	nd a A	nd a A	2.9 b A	1.5 a B	0.8 a B	3.5 b A	nd a A	0.8 a A	0.6 a A	0.6 a B	nd a A	7.6 b B
Caffeoylquinic acid	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
4,5-di-*O*-Caffeoylquinic acid	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
Caffeic acid derivative	nd a A	1.4 b B	nd a A	2.7 b B	nd a A	0.6 a A	2.4 b A	nd a	2.2 b A	5.5 a A	nd a	19.1 b B
di-Caffeic acid derivative	nd A	nd	nd	17.1 b B	nd a	nd a	nd A	nd	nd	4.6 b B	nd a	nd a
5-*p*-Coumaroylquinic acid	0.4 b A	0.3 a A	nd a A	1.5 c B	nd a A	0.7 b B	2.0 b A	0.1 a A	0.2 ab A	1.3 ab A	0.1 a A	3.4 b B
Ferulic acid	nd A	nd	nd	2.0 b B	nd a	nd a	nd	nd	nd	nd	nd	nd
1-*O*-Feruloylquinic acid	1.7 b A	nd a A	0.5 ab B	10.6 b B	0.9 a B	nd a A	2.6 b A	0.2 a A	1.9 ab A	2.0 a A	nd a A	1.4 a A
4-*O*-Feruloylquinic acid	nd A	nd	nd	2.4 b B	nd a	nd a	nd A	nd	nd	1.9 b B	nd a	nd a
5-*O*-Feruloylquinic acid	3.9 b A	1.3 ab B	nd a A	14.7 b B	nd a A	1.6 a A	6.9 b A	0.6 a A	0.5 a A	10.2 b A	nd a A	nd a A
Feruloylquinic acid	5.7 a A	13.0 a A	7.0 a A	7.7 a A	42.3 b B	26.6 ab B	2.0 a A	7.8 a A	1.4 a A	11.2 a A	15.3 a A	5.0 a A
Ferulic acid derivative	42.5 a A	36.5 a A	35.4 a A	56.0 b B	20.6 a A	21.0 a A	18.0 b A	6.9 a A	14.8 ab B	31.4 b A	6.1 a A	3.5 a A

	Phenolic content [mg (kg DM)$^{-1}$]											
	D			PL			D			PL		
	2008	2009	2010	2008	2009	2010	2008	2009	2010	2008	2009	2010
4-*O*-Feruloyl-5-*O*-caffeoylquinic acid	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
Caffeic / ferulic acid derivative	nd A	nd	nd	4.9 b B	nd a	nd a	nd	nd	nd	nd	nd	nd
Quercetin-3-*O*-galactoside	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
Total phenolics	**107.7 a A**	**77.6 a A**	**82.0 a A**	**189.6 b B**	**97.7 a A**	**90.3 a A**	**65.4 ab A**	**32.6 a A**	**84.3 b A**	**104.1 ab A**	**41.3 a A**	**134.7 b A**
Orange cultivars	‘Santa Cruz’						‘Nerac’					
Vanillic acid derivative	13.9 b B	5.4 a A	7.2 a B	8.5 a A	7.8 a A	5.8 a A	44.9 b B	18.5 a A	20.8 a B	21.2 b A	24.4 b A	11.7 a A
Vanillic acid hexoside	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
Tryptophan	20.3 b A	nd a A	nd a	38.7 c A	16.0 b B	nd a	12.5 b A	nd a	nd a	16.0 a A	nd a	nd a
p-Hydroxybenzoic acid hexoside	nd a	nd a	3.8 b B	nd	nd	nd A	nd a	nd a	2.6 b A	nd a	nd a	7.0 b B
3-*O*-Caffeoylquinic acid	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
5-*O*-*trans*-Caffeoylquinic acid	72.4 a A	19.6 a A	22.5 a A	174.3 a B	168.4 a B	310.1 b B	86.4 a A	35.6 a A	8.1 a A	110.9 a A	156.2 a B	183.9 a B
5-*O*-*cis*-Caffeoylquinic acid	3.5 a A	3.2 a A	4.1 a A	6.8 a B	179.9 b B	19.5 a B	2.9 a A	4.9 a A	1.5 a A	3.8 a A	9.2 b B	18.8 c B
Caffeoylquinic acid	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
4,5-di-*O*-Caffeoylquinic acid	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
Caffeic acid derivative	2.9 b A	nd a	nd a A	3.9 a A	nd a	60.9 b B	25.1 b A	nd a	nd a A	53.2 b A	nd a	58.1 b B
di-Caffeic acid derivative	32.8 a A	nd a	nd a	227.7 b B	nd a	nd a	31.6 a A	nd a	nd a	90.7 b A	nd a	nd a
5-*p*-Coumaroylquinic acid	5.2 a A	1.3 a A	2.7 a A	5.0 b A	nd a A	11.0 c B	0.8 a A	nd a A	0.6 a A	1.0 a A	1.2 ab B	2.0 b B
Ferulic acid	1.5 b A	nd a	nd a	3.0 b B	nd a	nd a	nd	nd	nd	nd	nd	nd
1-*O*-Feruloylquinic acid	17.8 b A	1.1 a B	1.7 a A	58.5 b B	nd a A	1.4 a A	nd a	0.9 a A	2.6 a A	nd a	3.0 b B	2.6 b A
4-*O*-Feruloylquinic acid	traces	nd	nd	traces	nd	nd	2.1 b A	nd a A	nd a	2.9 b A	0.8 a B	nd a
5-*O*-Feruloylquinic acid	20.3 b A	2.4 a B	3.4 a A	25.4 b A	nd a A	3.5 a A	26.9 b A	1.8 a A	3.6 a A	21.6 b A	5.6 a A	2.5 a A
Feruloylquinic acid	1.6 a A	16.8 b A	8.9 a A	16.9 a B	135.3 b B	44.3 a B	7.2 a A	41.7 b A	13.2 a A	10.7 a A	57.8 b A	63.0 b B
Ferulic acid derivative	41.7 b A	25.7 a B	18.4 a A	59.0 c B	10.6 a A	27.1 b A	39.8 b A	24.2 ab B	19.9 a A	61.0 b A	13.6 a A	16.3 a A
4-*O*-Feruloyl-5-*O*-caffeoylquinic acid	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
Caffeic / ferulic acid derivative	3.4 b A	nd a	nd a	22.4 b B	nd a	nd a	5.7 a A	nd a	nd a	17.5 b A	nd a	nd a
Quercetin-3-*O*-galactoside	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
Total phenolics	**237.2 b A**	**75.6 a A**	**72.6 a A**	**650.0 a B**	**517.9 a B**	**483.6 a B**	**285.8 a A**	**127.5 a A**	**72.9 a A**	**410.4 a A**	**271.8 a B**	**365.9 a B**
Red cultivars	‘Nutrired’						‘Pusa Kesar’					
Vanillic acid derivative	12.5 a A	9.4 a A	11.6 a B	13.1 b A	6.8 a A	8.3 a A	34.3 b B	11.8 a A	9.8 a B	10.3 b A	10.4 b A	5.9 a A
Vanillic acid hexoside	nd a	7.9 b A	0.8 a A	nd a	15.1 b B	1.0 a A	nd	nd	nd	nd	nd	nd
Tryptophan	23.7 b A	8.8 a A	nd a	106.5 b B	nd a A	nd a	124.9 a A	25.0 a A	0.7 a A	67.2 a A	44.1 a A	1.9 a B
p-Hydroxybenzoic acid hexoside	8.5 b A	nd a	5.3 b A	16.5 b B	nd a	7.8 b B	39.9 b A	9.9 a B	7.6 a A	39.2 c A	4.8 a A	9.7 b A
3-*O*-Caffeoylquinic acid	nd	nd	nd	nd	nd	nd	3.4 b B	nd a	nd a	1.0 b A	nd a	nd a
5-*O*-*trans*-Caffeoylquinic acid	29.3 b A	6.2 a A	64.7 c A	203.5 b B	66.9 a B	179.6 b B	584.0 b B	46.6 a A	55.2 a A	340.8 b A	417.7 b B	105.2 a B
5-*O*-*cis*-Caffeoylquinic acid	5.7 b A	0.5 a A	8.2 b A	12.0 b B	2.9 a B	14.1 b A	23.8 b B	5.1 a A	6.0 a A	13.0 a A	30.4 b B	8.4 a B
Caffeoylquinic acid	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
4,5-di-*O*-Caffeoylquinic acid	nd a	nd a	3.2 b A	nd a	nd a	14.5 b A	nd	nd A	nd	nd a	71.0 a A	nd a
Caffeic acid derivative	nd	nd	nd A	nd a	nd a	6.3 b B	135.9 b B	nd a	nd a	92.9 b A	nd a	nd a
di-Caffeic acid derivative	nd A	nd	nd	33.2 b B	nd a	nd a	224.9 b A	nd a	nd a	259.7 b A	nd a	nd a
5-*p*-Coumaroylquinic acid	2.0 a A	0.2 a A	1.0 a A	0.7 a A	nd a A	2.6 a A	23.3 b B	1.3 a A	1.4 a A	7.5 b A	1.1 a A	4.2 ab B
Ferulic acid	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
1-*O*-Feruloylquinic acid	8.9 b A	0.8 a A	2.8 a B	28.1 b B	1.4 a A	1.7 a A	nd a	4.7 b A	1.9 ab A	nd a	nd a A	2.2 b A
4-*O*-Feruloylquinic acid	1.8 b A	nd a	nd a	2.9 b A	nd a	nd a	4.5 a A	0.5 a A	nd a	1.0 b A	nd a A	nd a

	Phenolic content [mg (kg DM)$^{-1}$]											
	D			PL			D			PL		
	2008	2009	2010	2008	2009	2010	2008	2009	2010	2008	2009	2010
5-*O*-Feruloylquinic acid	29.4 b A	1.5 a A	5.2 a A	35.2 b A	1.2 a A	3.3 a A	69.2 b B	13.2 a A	8.7 a A	13.9 b A	nd a A	6.0 ab A
Feruloylquinic acid	5.7 a A	50.8 a A	26.7 a A	10.0 a B	286.8 c B	46.8 b A	24.5 a A	57.7 a A	63.2 a A	51.2 a A	169.4 b B	51.0 a A
Ferulic acid derivative	34.4 a A	45.1 a A	37.0 a B	45.3 a A	121.0 b B	12.2 a A	49.0 b B	12.0 a B	6.3 a A	23.1 b A	5.4 a A	5.1 b A
4-*O*-Feruloyl-5-*O*-caffeoylquinic acid	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
Caffeic / ferulic acid derivative	nd A	nd	nd	6.1 b B	nd a	nd a	27.6 a A	nd a	nd a	nd A	nd	nd
Quercetin-3-*O*-galactoside	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
Total phenolics	**161.9 a A**	**131.2 a A**	**166.4 a A**	**513.3 b B**	**502.1 b B**	**298.3 a B**	**1369.3 b B**	**187.6 a A**	**160.9 a A**	**920.7 b A**	**754.4 b B**	**199.5 a A**
Purple cultivars	'Anthonina'						'Deep Purple'					
Vanillic acid derivative	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
Vanillic acid hexoside	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
Tryptophan	40.8 b A	nd a	nd a	96.0 b A	nd a	nd a	35.6 b A	nd a	nd a	62.4 b A	nd a	nd a
p-Hydroxybenzoic acid hexoside	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
3-*O*-Caffeoylquinic acid	96.3 b A	40.8 a A	50.1 a B	256.7 b B	36.0 a A	27.8 a A	65.6 a A	43.0 a A	38.9 a A	122.9 b A	28.9 a A	75.7 ab A
5-*O*-*trans*-Caffeoylquinic acid	5268.4 a A	8498.2 b A	3279.3 a A	6969.6 b A	9212.4 b A	3073.6 a A	3565.7 a A	3167.4 a A	3433.3 a A	5146.9 ab A	6530.6 b B	2651.3 a A
5-*O*-*cis*-Caffeoylquinic acid	31.3 a A	105.0 b A	131.2 c A	28.8 a A	140.6 b A	168.5 b B	124.7 a A	106.8 a A	140.9 a A	141.0 a A	222.4 a B	122.3 a A
Caffeoylquinic acid	nd a	nd a	243.6 b B	nd a	nd a	165.3 b A	nd a	nd a	119.2 b A	nd a	nd a	103.1 b A
4,5-di-*O*-Caffeoylquinic acid	65.8 a A	31.7 a A	36.4 a B	65.1 b A	34.4 ab A	nd a A	14.6 a A	17.1 a A	29.7 a A	49.2 b A	27.8 ab A	nd a A
Caffeic acid derivative	500.9 c A	311.6 b A	94.3 a A	848.1 b B	236.4 a A	178.8 a A	211.2 a A	143.1 a A	225.8 a A	447.2 a A	253.0 a A	418.3 a A
di-Caffeic acid derivative	209.5 b A	nd a	nd a	475.6 b B	nd a	nd a	200.3 b A	nd a A	nd a	405.7 b A	24.0 ab B	nd a
5-*p*-Coumaroylquinic acid	7.1 a A	27.3 b B	12.9 a A	7.4 a A	8.9 a A	9.4 a A	11.4a B	30.9 b B	11.9 a B	6.2 a A	9.3 a A	6.9 a A
Ferulic acid	96.6 b A	1.8 a A	15.8 a B	114.4 b A	1.7 a A	nd a A	21.2 ab A	34.9 b B	16.5 a B	98.1 b B	4.8 a A	nd a A
1-*O*-Feruloylquinic acid	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
4-*O*-Feruloylquinic acid	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
5-*O*-Feruloylquinic acid	24.9 b A	10.6 a A	7.5 a A	57.3 b B	8.8 a A	7.8 a A	0.6a A	0.4 a B	0.1 a A	2.1 a A	0.1 a A	0.2 a A
Feruloylquinic acid	nd a	39.7 b A	85.9 c A	nd a	28.0 a A	242.6 b B	nd a	12.0 a A	183.9 b A	nd a	57.0 a B	255.0 b A
Ferulic acid derivative	2118.1 b A	1980.4 b A	1292.4 a A	2079.9 b A	1911.9 b A	880.8 a A	2387.9 a A	1850.8 a A	1207.8 a B	1985.5 b A	2404.1 b A	480.3 a A
4-*O*-Feruloyl-5-*O*-caffeoylquinic acid	49.1 a A	83.8 a A	82.6 a B	42.1 ab A	65.3 b A	nd a A	8.0 a A	35.0 b A	nd a	143.9 c B	73.0 b B	nd a
Caffeic / ferulic acid derivative	111.3 b A	nd a	nd a	256.2 b B	nd a	nd a	135.8 b A	nd a	nd a	413.1 b A	nd a	nd a
Quercetin-3-*O*-galactoside	58.3 a A	52.6 a A	52.0 a B	67.2 c A	53.2 b A	nd a A	43.8 a A	52.2 a A	47.6 a B	57.9 b A	70.3 b B	nd a A
Total phenolics	**8678.2 b A**	**11183.4 c A**	**5383.9 a A**	**11364.5 b B**	**11737.4 b A**	**4754.7 a A**	**6826.4 a A**	**5493.7 a A**	**5455.6 a A**	**9082.1 b A**	**9705.3 b B**	**4113.0 a A**

nd - not detected
abc Values derived from samples within one location and different cultivation years with different lower case letters are significantly different
ABC Values derived from samples of different locations within one cultivation year with different upper case letters are significantly different

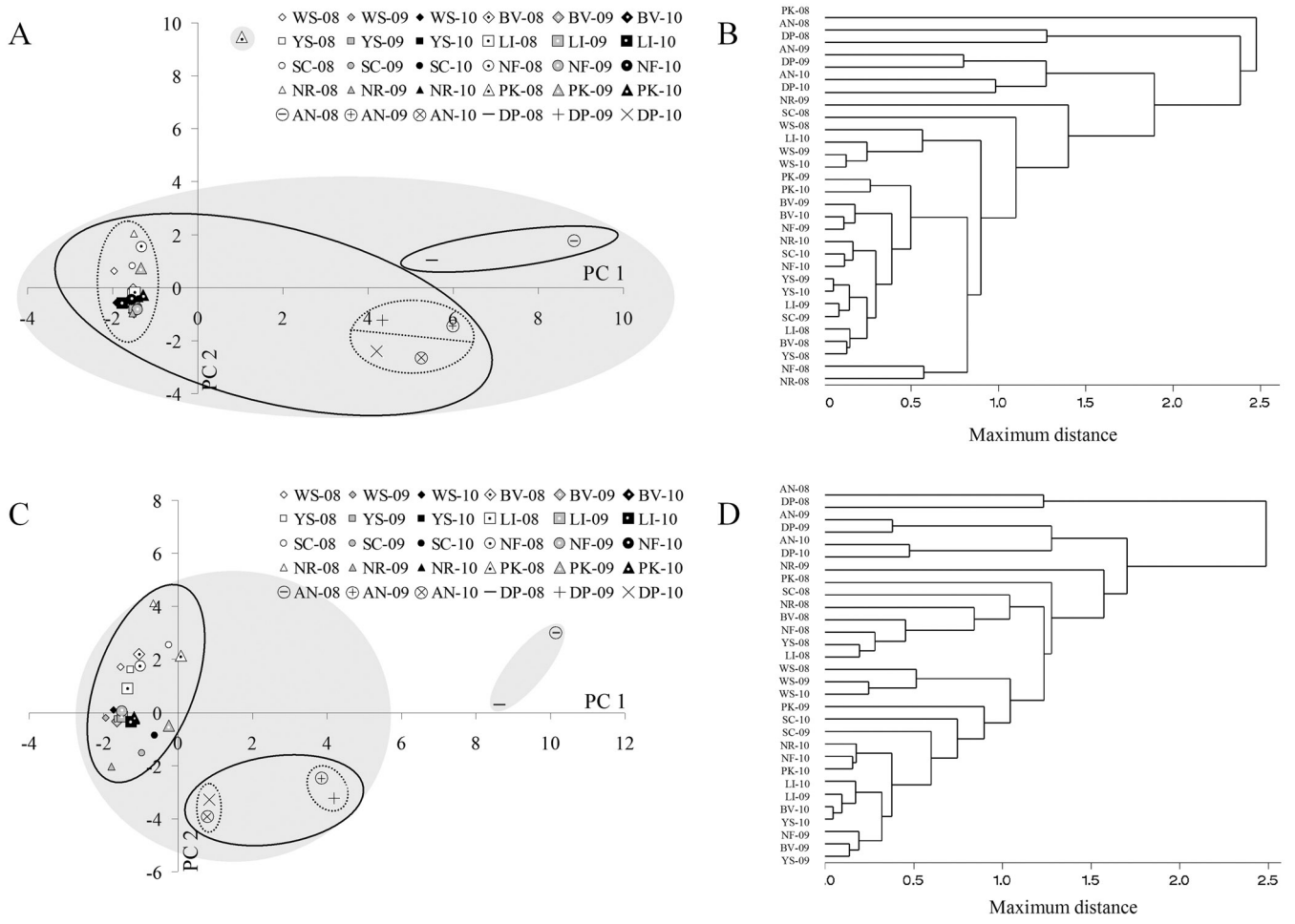

Fig. 2: Influence of cultivation year on phenolic compounds of different carrot cultivars grown at location D (A+B) and PL (C+D): (A/C) PCA scatter plots of the first two principal components (PC 1/PC 2) and (B/D) dendrograms of complete-linkage cluster analysis. Main clusters and their secondary and tertiary subgroups shown by cluster analysis are indicated in the PCA plots (A/C) by light-grey areas, black-rimmed and black-dotted ellipses, respectively.

those of 'Deep Purple'. Moreover, for 2009 the growing locations of the latter were grouped into different clusters (Fig. 3). Considering the other cultivars, for 2008 the values of 'Pusa Kesar' of both growing locations could be distinguished from other white, yellow, orange, and red cultivars. Interestingly, in 2010 the means of both red cultivars 'Pusa Kesar' and 'Nutrired' scored in a tertiary subgroup. However, the location did not have any influence on this subgroup.

Total phenolic contents of roots grown in PL and D varied in a wide range. Remarkably, in PL higher total phenolic contents were found for most carrot roots, except for 'Pusa Kesar' grown in 2008 as well as 'Anthonina' and 'Deep Purple' cultivated in 2010. Although there was a great variability, the variation among cultivars did not differ notably between both growing locations within the years (Tab. 3).

Discussion

The aim of this study was to clarify the role of genotype and environment (growing location and cultivation year) on the phenolic contents of carrot cultivars exposed to European growing conditions. In summary, 20 phenolic compounds have been identified in carrot roots. Proof has been furnished that there was a broad variation throughout differently coloured cultivars, while purple carrots behaved differently. In accordance with the findings of Alasalvar et al. (2001) purple roots accumulated highest amounts of total phenolics, only comprising hydroxycinnamic acids, with 5-*O*-*trans*-caffeoylquinic acid being the predominant compound (Kreutzmann et al., 2008; Sun et al., 2009). Assuming a dry matter content of approx. 10%, the contents in purple carrot roots were on the same level as reported in previous studies (Alasalvar et al., 2001; Kammerer et al., 2004; Kreutzmann et al., 2008). Results of PCA and CA support the outstanding position of purple cultivars and hence the strong influence of genotype (Fig. 2 and 3). Also the cultivation year showed an effect on purple roots, since the values of year 2008 could clearly be separated from those of 2009 and 2010 (Fig. 2). However, this may also partly be due to different sample preparations after harvest (see Material and methods). In contrast, growing location did not exert such a clear-cut influence on polyphenol contents. In 2010, the differences between both locations (Fig. 2) may be explained by differing water supply (Tab. 1).

In carrot cultivars being devoid of anthocyanins (white, yellow, orange, and red roots), considerably lower total phenolic contents, comprising hydroxycinnamic and hydroxybenzoic acids, were observed, which was also reflected by chlorogenic acid amounts. Despite the great variations of total phenols between the cultivars, differences were insignificant as also previously reported by Sun et al. (2009), supposedly due to the heterogeneity of sample material within the cultivars (Mattila and Hellström, 2007). However, based on PCA and CA results, carrot 'Pusa Kesar' (red) was grouped in a separated position in 2008, whereas in 2010 the values of both

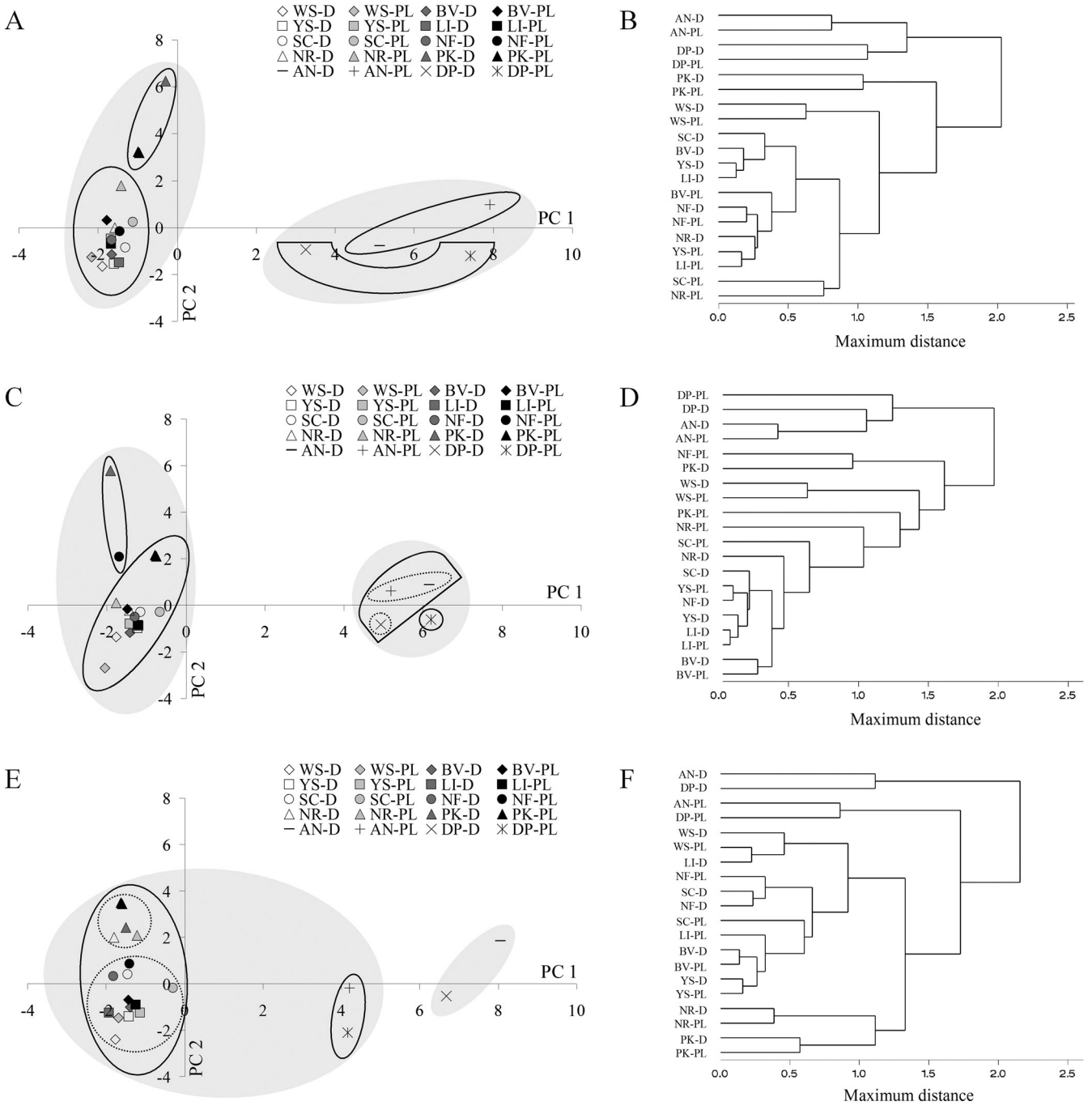

Fig. 3: Influence of location on phenolic compounds of different carrot cultivars grown in 2008 (A+B), 2009 (C+D), and 2010 (E/F): (A/C/E) PCA scatter plots of the first two principal components (PC 1/PC 2) and (B/D/F) dendrograms of complete-linkage cluster analysis. Main clusters and their secondary and tertiary subgroups shown by cluster analysis are indicated in the PCA plots (A/C) by light-grey areas, black-rimmed and black-dotted ellipses, respectively.

red cultivars 'Pusa Kesar' and 'Nutrired' differed from the other roots. Again, influence of cultivation year and growing location was of minor relevance.

Consequently, the results of the present study support the effects of cultivar to be predominant, although solely purple cultivars were found to significantly differ when compared with further differently coloured genotypes. In agreement with previously published data, considerable variability was found among carrot cultivars regarding their phenolic contents, in particular in purple carrots (ALASALVAR et al., 2001; KREUTZMANN et al., 2008; METZGER and BARNES, 2009; NICOLLE et al., 2004; SUN et al., 2009). Consistently, also for different orange carrot genotypes it has been shown that much of the variation in phenolic acids can be attributed to the genetic diversity among cultivars (BOZALAN and KARADENIZ, 2011; TALCOTT and HOWARD, 1999a). Moreover, SIMON et al. (1982) reported that beside phenolics various other attributes such as flavour attributes, total sugars, carotenoids, or total terpenoids are known to be rather influenced by carrot genotype than by climate or soil.

TALCOTT and HOWARD (1999a) described a considerable influence of different growing areas on phenolic compounds in carrots, assuming adverse growing conditions or improper post-harvest handling to be responsible for this effect. In contrast, the observed location

influence in the present study was found to be minor, since phenolic variation among cultivars in different years was comparable at both locations. This may be due to similar climatic conditions (Tab. 1), as Kraków (PL) and Quedlinburg (D) are situated on adjoining latitudes, and also identical harvesting practices were used. The year-to-year variations between 2008 and 2009 / 2010, especially observed in purple roots, may have resulted from different sample processing after harvest as mentioned above. Therefore, we suggest the influence of cultivation year to be of minor relevance, because the climatic conditions, which were considered to be of major influence on polyphenol content (MANACH et al., 2004) did not vary significantly between the years of observation. Also SØLTOFT et al. (2010) did not find significant year-to-year variation of phenolics in carrots grown at one location in different cultivation systems.

In conclusion, the results demonstrate a variation in phenolic compounds between differently coloured carrot cultivars. In particular, purple cultivars could be clearly distinguished from cultivars being devoid of anthocyanins without using their anthocyanin contents for analysis. When grown on different locations having similar climatic conditions, variations of phenolic patterns are marginal. Therefore, selection of cultivar for a given commodity is an important factor in providing raw material with specified phenolic patterns. Thus, these results provide an indication for breeders and the processing industry. However, further investigations are required to compile a more comprehensive database considering different environmental conditions such as temperature, water, and light. Furthermore, due to their outstanding phenolic contents, our findings suggest the inclusion of purple carrot into human diets to exploit their putative health beneficial effects.

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