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Anthocyanin Composition of Black Carrot (*Daucus carota* ssp. *sativus* var. *atrorubens* Alef.) Cultivars Antonina, Beta Sweet, Deep Purple, and Purple Haze

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ABSTRACT: This study aimed to identify the pigment composition of black carrot (*Daucus carota* ssp. *sativus* var. *atrorubens* Alef.) cultivars Antonina, Beta Sweet, Deep Purple, and Purple Haze. Cyanidin 3-xylosyl(glucosyl)galactosides acylated with sinapic acid, ferulic acid, and coumaric acid were detected as major anthocyanins by high-performance liquid chromatography with diode array detection (HPLC-DAD) and with electrospray ionization multiple mass spectrometry (HPLC-ESI-MS^n) analyses. The preparative isolation of these pigments was carried out by means of high-speed countercurrent chromatography (HSCCC). The color activity concept was applied to the isolated anthocyanins at three pH values. Cyanidin 3-xylosyl(sinapoylglucosyl)galactoside was found to exhibit a lower visual detection threshold and a higher pH stability than cyanidin 3-xylosyl(feruloylglucosyl)galactoside and cyanidin 3-xylosyl(coumaroylglucosyl)galactoside. The color parameters of the fresh roots of the four cultivars were described by the CIELab coordinates L^* (lightness), C^* (chroma), and h_{ab} (hue angles). Total phenolics varied among the cultivars and ranged from 17.9 to 97.9 mg gallic acid equivalents (GAE)/100 g fresh weight (fw). For the content of monomeric anthocyanins, values between 1.5 and 17.7 mg/100 g fw were determined.

KEYWORDS: Anthocyanins, *Daucus carota*, high-speed countercurrent chromatography, CIELab

INTRODUCTION

Black or purple carrots (*Daucus carota* ssp. *sativus* var. *atrorubens* Alef.) originate from Turkey and the Middle and Far East, where they have been cultivated for at least 3000 years.[1] They have an attractive bluish-purple color with high levels of anthocyanins and can serve as a natural food colorant due to their high heat, light, and pH stability.[2] In recent years, many new varieties with an extremely high content of pigments were cultivated. The anthocyanin content of several of these black carrot cultivars was reported to be in the range of up to 1750 mg/kg fresh weight.[3] Today extracts of black carrots are commonly used in juices, candies, confectionery, ice cream, soft drinks, or other fermented beverages as a healthier alternative to synthetic colorants like FD&C Red 40.[4,5]

Black carrots mainly consist of cyanidin-based pigments, and their anthocyanin profile was analyzed by several investigators.[1,6] The structure of the two nonacylated anthocyanins cyanidin 3-xylosyl(glucosyl)galactoside and cyanidin 3-xylosylgalactoside, as well as the three monoacylated anthocyanins cyanidin 3-xylosyl(sinapoylglucosyl)galactoside, cyanidin 3-xylosyl(feruloylglucosyl)galactoside, and cyanidin 3-xylosyl(coumaroylglucosyl)galactoside were characterized by NMR analyses.[7,8] Kammerer et al.[6] showed that the acylated anthocyanins in different black carrot varieties ranged from 55% to 99% of the total anthocyanin content, and it was shown that these acylated cyanidin derivatives are more stable during prolonged storage compared to the nonacylated ones.[2] Up to now, there are only few reports on the isolation of anthocyanins from black carrots.[9,10] The main objective of this work was the development of a method for preparative isolation of anthocyanins from the black carrot varieties Antonina, Beta Sweet, Deep Purple, and Purple Haze involving high-speed countercurrent chromatography (HSCCC). HSCCC is a modern automated liquid−liquid chromatographic technique that has been used for the preparative isolation of numerous natural products because of the gentle working conditions and large yields of pure compounds.[11−13] Due to an increasing demand for natural colorants by food industry, studies in alternatives to synthetic food additives like our research are of particular relevance from a scientific and economic viewpoint. With the isolated anthocyanins, it will be possible to determine the color contribution of individual compounds by applying the color activity concept.[14]

The four different cultivars were also evaluated in terms of total polyphenols, monomeric anthocyanins, qualitative anthocyanin composition, and color properties. The colors of the fresh carrots were described by CIELab coordinates for the four cultivars. Color parameters included lightness (L^*), chromatic tonality (hue angle, h_{ab}), and metric chroma (C^*) measured in the CIELab scale.

MATERIALS AND METHODS

Plant Material. The black carrot varieties Antonina, Deep Purple, Beta Sweet, and Purple Haze were sown and grown in the agricultural area of the University of Applied Sciences, Osnabrück (Germany), in 2010. The supplier for black carrot seeds Purple Haze and Deep Purple was Bejo Zaden (bj) from Netherlands; Beta Sweet was provided by Transimpex, and Antonina was provided by Sperli, a local supplier.

Solvents and Reagents. All reagents and solvents employed were of HPLC purity or analytical grade. Methanol and *tert*-butyl

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methyl ether (TBME) were redistilled prior to use. Trifluoroacetic acid (TFA) was obtained from Sigma−Aldrich (Munich, Germany); deuterated NMR solvents were from Deutero (Kastellaun, Germany); and formic acid p.A., >98%, was from Acros Organics (Belgium).

Extraction of Anthocyanins. Pigment extraction was carried out according to a method previously described by Eichhorn and Winterhalter [15] and Hillebrand et al.[12] Approximately 900 g of black carrot slices was blanched at 100 °C for 3 min with 1 L of demineralized water. The same volume of an aqueous hydrochloric acid solution (19/1 v/v) was added. The suspension was cooled at 0 °C for 3 h and then stored at room temperature for 8 h without stirring. To remove solid material, the suspension was filtered prior to application onto an Amberlite XAD-7 column (Fluka, 100 × 7 cm), conditioned with 2 L of methanol and then with 2 L of water. The column was rinsed with 3 L of water to remove sugars, proteins, organic acids, and minerals, and anthocyanins were eluted with 2 L of a mixture of methanol/glacial acetic acid (19/1 v/v). The eluate was concentrated in vacuo, dissolved in water, and freeze-dried. Between 2.5 and 3.1 g of purified XAD-7 extract was obtained from 900 g of fresh material.

Subsequent isolation of pigments was achieved by high-speed countercurrent chromatography. Separations were carried out with a high-speed model CCC-1000 (Triplecoil; diameter of tubing 2.6 mm, total volume 850 mL, revolution speed 850 rpm) produced by Pharma-Tech Research Corp. (Baltimore, MD). A two-phase solvent system, consisting of *tert*-butyl methyl ether/*n*-butanol/acetonitrile/water (2/2/1/5 v/v/v/v, acidified with 0.1% trifluoroacetic acid) was used (less dense layer as stationary phase) with a flow rate of 4 mL/min. An amount of 500 mg of each XAD-7 extract (redissolved in 22 mL of solvent mixture) was injected for a single run, and all CCC fractionations were monitored at 520 nm. The isolated pure pigments were converted into chloride salts by adding an equimolar amount of hydrochloric acid.

High-Performance Liquid Chromatography. HPLC analyses were performed on a MD-910 multiwavelength detector (wavelength range between 220 and 650 nm), equipped with a DG-980-50 three-line degasser and a LG-980-02 ternary gradient unit, a PU-980 Intelligent HPLC pump, an AS-950 Intelligent autosampler, and Borwin PDA chromatography software (Jasco, Gross-Umstadt, Germany). HPLC separation was carried out on Luna RP-18 column (250 × 4.6 mm, 5 μm, Phenomenex, Aschaffenburg, Germany) at a flow rate of 0.5 mL/min, with an injection volume of 20 μL (in solvent system A). The binary gradient consisted of solvent system A (water/acetonitrile/formic acid 87/3/10 v/v/v) and solvent system B (water/acetonitrile/formic acid 40/50/10 v/v/v). Conditions were as follows: 0 min, 6% B; 20 min, 20% B; 35 min, 40% B; 40 min, 60% B; 45 min, 90% B; and 55 min, 6% B; followed by a 5 min equilibration period.

High-Performance Liquid Chromatography−Electrospray Ionization Multiple Mass Spectrometry. Freeze-dried samples (XAD-7 extracts as well as CCC fractions containing a mixture of anthocyanins) were redissolved in a mixture of water/acetonitrile/formic acid, 95/3/2 (v/v/v), and were analyzed by HPLC−ESI-MS^n. HPLC analyses were performed on an Agilent HPLC system (Böblingen, Germany) equipped with a binary pump (1100 series) and an autosampler (1200 series). The same analytical conditions as described above were used. ESI-MS^n measurements were performed on a Bruker Esquire-LC multiple ion trap mass spectrometer (Bruker Daltonics, Bremen, Germany). Mass spectral analyses were recorded under the following conditions: positive ion mode; capillary, −2500 V; capillary exit offset, 70 V; end plate offset, −500 V; skimmer 1, 20 V; skimmer 2, 10 V; dry gas, N_2 at 11 L/min; dry temperature, 325 °C; nebulizer, 60 psi; scan range, 50−2200 *m*/*z*. Esquire NT 4.0 software (Bruker Daltonics, Bremen, Germany) was used for analysis and data collection.

Electrospray Ionization Multiple Mass Spectrometry. For ESI-MS^n experiments, pure pigments were redissolved in a mixture of water/acetonitrile/formic acid, 95/3/2 (v/v/v), and were delivered directly via a syringe pump 74900 (Cole-Parmer) into the ESI source (flow rate 240 μL/h). Mass spectrometry parameters were the following: positive ion mode; capillary, −3500 V; capillary exit offset, 60 V; end plate offset, −500 V; skimmer 1, 30 V; skimmer 2, 10 V; dry gas, N_2 at 4 L/min; dry temperature, 300 °C; nebulizer, 10 psi; scan range, 50−1000 *m*/*z*.

Proton and Carbon Nuclear Magnetic Resonance Spectroscopy. ^{1}H and ^{13}C NMR measurements were performed on a Bruker AMX 600 spectrometer (Bruker Biospin, Rheinstetten, Germany) at 600.13 and 150.92 MHz, respectively. Pure pigments were dissolved in a mixture of methanol-d_4/TFA-d_1 (19/1 v/v), and data were processed by WIN-NMR software version 6.1.0 and were compared to literature.[7,8]

Quantification of Total Phenolics and Anthocyanins. For determination of phenolics as well as monomeric anthocyanin content, black carrots were washed with water and cut into small pieces with a vegetable slicer. An amount of 40 mL of a mixture of ethanol/water (80/20 v/v) was added to approximately 10 g of carrot slices and the suspension was treated by an Ultra Turrax apparatus for 1 min. After the black carrot pieces were separated from the extract by filtration into a volumetric flask, the residue was washed with a 20 mL portion of formic acid (2%) and the final volume was adjusted to 100 mL by addition of formic acid (2%).

Total phenolic content in the extracts was determined with Folin−Ciocalteu reagent[16] and gallic acid as standard. Total phenolic values were reported in milligrams gallic acid equivalents per 100 g fresh weight (mg GAE/100 g fw). For quantification of anthocyanins, a five-point calibration curve of cyanidin 3-glucoside (3.2−50.8 mg/L) was used. All anthocyanins were detected at 520 nm and were calculated as cyanidin 3-glucoside equivalents. Values were means ± SD of three independent experiments.

Color Analysis. Aqueous citric acid (0.1 M) solutions (pH 3.5) were colored with 15, 30, and 45 mg anthocyanin/100 mL from the black carrot (Antonina, Beta Sweet, Deep Purple, and Purple Haze) anthocyanin extracts mentioned above for quantification, with monomeric anthocyanin contents of 17.7, 8.1, 12.5, or 1.5 mg/100 mL, respectively. Solutions were prepared in triplicate. The absorbance spectra were measured by a double-beam spectrophotometer (Jasco, Gross-Umstadt, Germany), using 0.1 cm path length cells. Measurements were taken every 1 nm between 380 and 780 nm. A slit width of 2.0 nm was used for all determinations. All samples had been clarified by centrifugation and filtration through a 0.45 μm membrane (Macherey-Nagel, Düren, Germany). Nanopure water (Barnstead) was used as blank, and absorbances were corrected to 1 cm path length. From the spectra, the rectangular coordinates L^*, a^*, and b^* and the cylindrical coordinates CIE C^* and h^* were calculated by the CIE method,[17] with the 10° standard observer and the illuminant D65.

Determination of Visual Detection Threshold (VDT). A 1−2 mg portion of each of the purified anthocyanins was dissolved in 5 mL of McIlvaine buffer containing 0.1 M citric acid solution (solution A) and 0.2 M disodium hydrogen phosphate solution (solution B). Depending on the pH value, the amounts of the two solutions A and B were combined in different volumes covering a range from pH 2 to pH 5.[18] By further dissolving the anthocyanin

Table 1. Total Phenolic and Total Monomeric Anthocyanin Content of Different Black Carrot Varieties

cultivar	total phenolics (mg GAE/100 g fw)[a]	anthocyanins[b] (mg/100 g fw)
Antonina	75.3 ± 9.8	17.7 ± 1.9
Beta Sweet	28.5 ± 1.4	8.1 ± 1.8
Deep Purple	97.9 ± 3.2	12.5 ± 1.5
Purple Haze	17.9 ± 1.4	1.5 ± 0.3

[a] GAE, gallic acid equivalents; fw, fresh weight. [b] Calculated as cyanidin 3-glucoside equivalents.

stock solution (1:100, 1:150, 1:200, etc.), a dilution series was obtained and exactly 4 mL of each anthocyanin solution was filled into a plastic cuvette (width 1 cm). All cuvettes were placed in order of increasing dilution factor in bright white styrofoam trays, whereas the sample cuvettes were separated by cuvettes filled with the corresponding buffer. By looking onto the cuvettes, at least three test persons determined independently the concentration at which a color impression can be last observed and visual detection threshold is calculated as milligrams per liter and micromoles per liter, respectively.

RESULTS AND DISCUSSION

Total Phenolics and Total Monomeric Anthocyanins. Concentration of phenolic compounds was examined by the Folin−Ciocalteu assay and calculated as gallic acid equivalents (GAE). Phenolic content varied significantly among the four black carrot cultivars, ranging from 17.9 to 97.9 mg GAE/100 g fresh weight (fw). These values are much higher than the values reported for yellow (7.7 mg/100 g fw) and white carrots (8.69 mg/100 g fw).[19]

The amount of phenolic compounds in roots of Deep Purple (97.9 mg GAE/100 g fw) was slightly higher than in Antonina (75.3 mg GAE/100 g fw), and considerably higher in comparison with the Beta Sweet (28.5 mg GAE/100 g fw) and Purple Haze (17.9 mg GAE/100 g fw) cultivars. The quantitative HPLC-DAD analysis revealed a remarkable variation in anthocyanin content, ranging from 1.5 to 17.7 mg/100 g fw. The cultivars Antonina, Deep Purple, and Beta Sweet contained higher levels of anthocyanins than the cultivar Purple Haze, which was already visually indicated by the degree of root coloring.

These anthocyanin values were comparable to those reported earlier for several genotypes of red-fleshed potatoes.[20] The anthocyanin contents for black carrot were lower than those reported for black currant and blueberries (476−365 mg/100 g fw), but they were similar to other foods such as plum (19−124 mg/100 g fw), strawberries (21−40 mg/100 g fw), red onion (49 mg/100 g fw), and red grapes (27 mg/100 g fw).[21] The contents of phenolic compounds and monomeric anthocyanins are listed in Table 1.

Determination of Anthocyanin Composition. The anthocyanin composition of black carrots consists mainly of mono- and nonacylated derivatives. The HPLC-DAD chromatograms (520 nm) of anthocyanins extracted from the four tested cultivars are shown in Figure 1. With the HPLC conditions used in this study, at least 10 peaks were separated from the anthocyanin extracts of the four genotypes analyzed. The chromatographic characteristics and mass spectrometric properties of the separated compounds are shown in Table 2. Antonina and Deep Purple cultivars showed 7 and 6 anthocyanin compounds, respectively, with the major peaks consisting of cyanidin 3-xylosyl-galactoside **2**, cyanidin 3-xylosyl(feruloylglucosyl)galactoside **5**, and cyanidin 3-xylosyl(coumaroylglucosyl)galactoside **6** (Table 2). The most abundant pigments in the varieties Beta Sweet and Purple Haze are cyanidin 3-xylosyl(glucosyl)galactosides acylated with sinapic acid **4**, ferulic acid **5**, and coumaric acid **6**, respectively. Figure 2 shows the structures of the characterized and isolated acylated pigments. The variety Beta Sweet showed two major and one minor pigment by HPLC analysis. Peaks **4** and **5** represented about 43.5% and 38.6% of the total area at 520 nm and were identified as cyanidin 3-xylosyl(sinapoylglucosyl)galactoside (**4**) and cyanidin 3-xylosyl(feruloylglucosyl)galactoside (**5**), respectively. The minor pigment **6** was identified by HPLC-ESI-MS^n analysis as cyanidin 3-xylosyl(coumaroylglucosyl)galactoside. Peak **9** was one of the later-eluting compounds that has not been identified in previous reports on anthocyanins in black carrot roots. By means of HPLC-ESI-MS^n analysis, compound **9** was tentatively identified as peonidin 3-xylosyl(sinapoylglucosyl)galactoside.

Through calculation of percentage peak area from HPLC measurement, the pigments cyanidin 3-xylosyl(feruloylglucosyl)galactoside (**5**) and cyanidin 3-xylosyl(sinapoylglucosyl)galactoside (**4**) accounted for 44.6% and 25.6% of the total area at 520 nm, and therefore they are the predominant anthocyanins of the cultivar Purple Haze. Table 3 presents retention time, percentage peak area, and UV−vis absorbance data of the characterized major anthocyanins. The λ_{max} of the monoacylated pigments **4**−**6** were at 525 and 528 nm, respectively. Furthermore, they exhibited an additional peak at 323−332 nm, thus indicating that these anthocyanins are acylated with hydroxycinnamic acids.

Isolation of Pigments by High-Speed Countercurrent Chromatography. Identification of individual anthocyanin compounds was primarily based on the data from HPLC-DAD−ESI-MS^n analyses, by comparison with literature data of black carrot anthocyanins.[1,6−8] HPLC-DAD−ESI-MS^n technique was found to be an excellent tool for anthocyanin characterization, due to its highly sensitive ionization producing intact pseudomolecular ions and the corresponding anthocyanidin fragments. For a more thorough characterization of the pigments, isolation by high-speed countercurrent chromatography (HSCCC) was carried out. In the following, as an example, the HSCCC separation of the variety Purple Haze is shown. Purple Haze is a hybrid carrot, with strong growth, slightly fibrous texture, and a sweet flavor. By means of high-speed countercurrent chromatography, four fractions as well as the coil residue were obtained. CCC fraction 2 (23 mg) contained the acylated anthocyanin cyanidin 3-xylosyl(sinapoylglucosyl)galactoside (**4**) in a purity of 85%. Upon HPLC−ESI-MS^n analysis, a pseudomolecular ion $[M]^+$ at m/z 949 was obtained. The first fragment ion was generated by loss of one xylose moiety (132 amu). The loss of 530 amu can be ascribed to the presence of a disaccharide moiety (Glc-Gal) acylated with sinapic acid. These findings are in accordance with data reported for an Afghan black carrot and a wild carrot variety.[7,8] CCC fraction 3 (30 mg) contained cyanidin 3-xylosyl-(feruloylglucosyl)galactoside (**5**) in a purity of 92%, which is the major pigment of the Purple Haze cultivar. The structure of **5** was

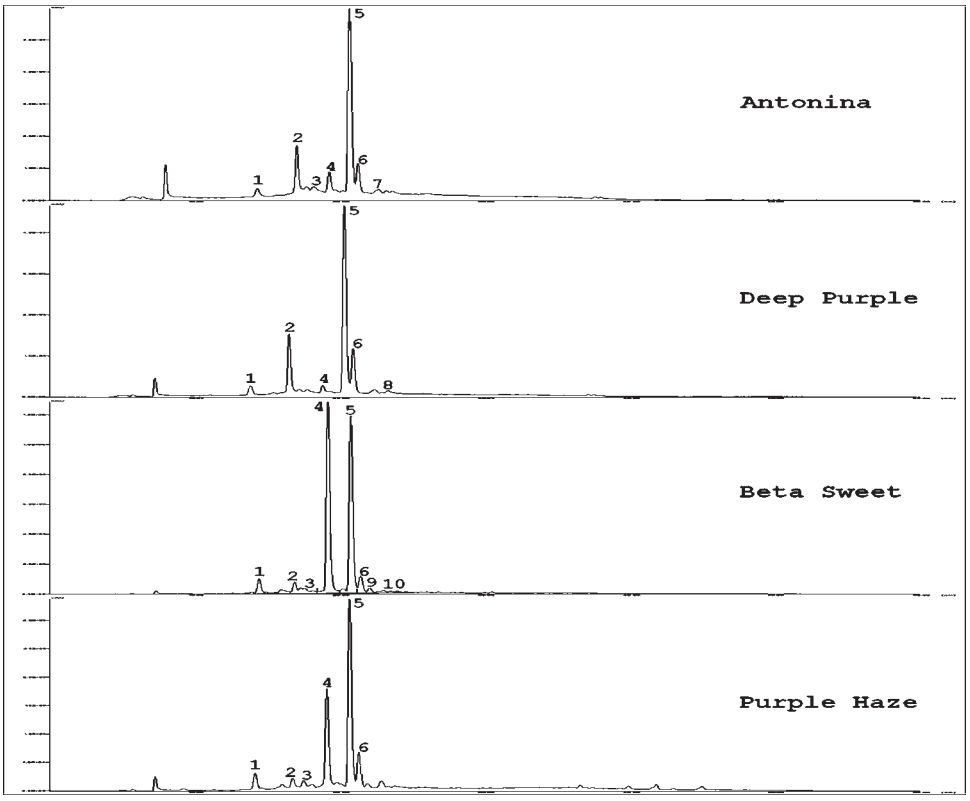


Figure 1. HPLC chromatograms of the anthocyanin-enriched XAD-7 extracts of black carrot cultivars Antonina, Deep Purple, Beta Sweet, and Purple Haze at 520 nm. For peak numbering cf. Table 2.

Table 2. Mass Spectrometric Data and Identification of Anthocyanin Compounds Characterized from Different Black Carrot Cultivars

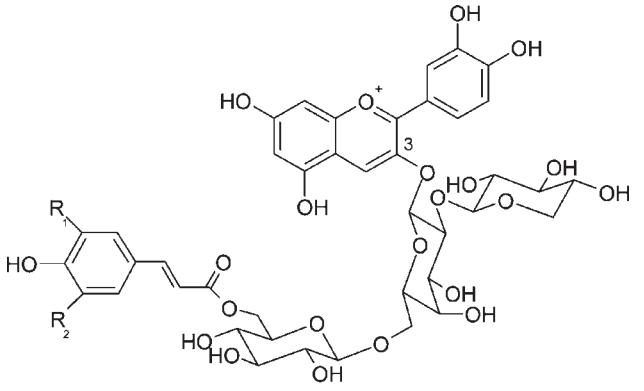
		m/z			
peak[a]	retention time (min)	M^+	aglycon	compound identity	refs
1	13.5	743	287	cyanidin 3-xylosyl(glucosyl)galactoside	7, 8, 24
2	16.2	581	287	cyanidin 3-xylosylgalactoside	7, 8, 24
3	16.7	863	287	cyanidin 3-xylosyl(*p*-hydroxybenzoylglucosyl)galactoside	24
4	18.4	949	287	cyanidin 3-xylosyl(sinapoylglucosyl)galactoside	7, 8, 24
5	19.8	919	287	cyanidin 3-xylosyl(feruloylglucosyl)galactoside	7, 8, 24
6	20.4	889	287	cyanidin 3-xylosyl(coumaroylglucosyl)galactoside	7, 8, 24
7	21.8	903	271	pelargonidin 3-xylosyl(feruloylglucosyl)galactoside	1
8	22.3	595	301	peonidin 3-xylosylgalactoside	1
9	22.7	963	301	peonidin 3-xylosyl(sinapoylglucosyl)galactoside	*b*
10	23.3	933	301	peonidin 3-xylosyl(feruloylglucosyl)galactoside	1

[a] Peaks are shown in Figure 1. [b] Tentatively identified in this work (cf. text).

Table 3. Chromatographic Properties of Acylated Anthocyanins Detected in Black Carrot Varieties by HPLC-DAD Analysis

			peak area[a] (%)			
peak label[b]	retention time (min)	λ_{max} (nm)	Beta Sweet	Purple Haze	Antonina	Deep Purple
4	18.4	528, 332, 282	43.5	25.6	6.2	3.1
5	19.8	525, 329, 282	38.6	44.6	35.3	45.3
6	20.4	525, 323, 282	4.7	11.9	8.13	13.1

[a] Relative area. [b] For compound labeling, cf. Table 2 and Figure 1.



compd[a]	R_1	R_2
4	OCH_3	OCH_3
5	OCH_3	H
6	H	H

Figure 2. Structures of major acylated anthocyanins found in black carrot. [a]For compound labeling cf. Table 2.

confirmed by HPLC−ESI-MS^n measurements. The pseudomolecular ion $[M]^+$ with m/z 919 yielded fragments of m/z 625 and 287, respectively (Table 2). Approximately 10 mg of cyanidin 3-xylosyl(coumaroylglucosyl)galactoside (**6**) could be isolated from fraction 4 in a purity of 96%. Mass spectrometric analysis of **6** showed a molecular ion $[M]^+$ at m/z 889 and fragments at m/z 581 and 287, respectively. HPLC-ESI-MS^n spectra were compared with literature data previously recorded by Kammerer et al.[1,6] Several milligrams of pure anthocyanins were obtained within a single run by HSCCC, thus allowing them to be used for NMR spectroscopic characterization, which was in accordance with literature data,[8] and further studies on bioactivity, bioavailability, metabolism, as well as color activity measurements.

Determination of Visual Detection Thresholds at Different pH Values. In order to determine the visual detection threshold of pure pigments, the color activity concept[14] was applied. Evolving from flavor research, this concept defines the visual detection threshold of a substance as its concentration at which a color impression is just visible and is calculated by dividing the concentration of the analyzed pigment by the factor of dilution.[22,23] The visual detection thresholds of the three acylated anthocyanins isolated by HSCCC separations were evaluated under different pH conditions. At pH 3.0 the acylation of cyanidin 3-xylosyl(glucosyl)galactoside with sinapic acid, ferulic acid, and coumaric acid results in a visual detection threshold of 0.09 mg/L for cyanidin 3-xylosyl(sinapoylglucosyl)galactoside (**4**), 0.11 mg/L for cyanidin 3-xylosyl(feruloylglucosyl)galactoside (**5**), and 0.15 mg/L for cyanidin 3-xylosyl(coumaroylglucosyl)galactoside (**6**). In all cases, visual detection thresholds (VDT) increased when pH was raised and substitution of cyanidin 3-xylosyl-(glucosyl)galactoside showed a lower color loss at pH 5.0 with sinapic acid (VDT = 0.15 mg/L) than with ferulic acid (VDT = 0.30 mg/L) and coumaric acid (VDT = 0.32 mg/L). Possibly, an increasing methoxylation of cinnamic acid residues protects the flavylium cation from nucleophilic attack at higher pH values. These results show that slightly different substitution of cinnamic acid residues affects a protection of the chromophoric structure of

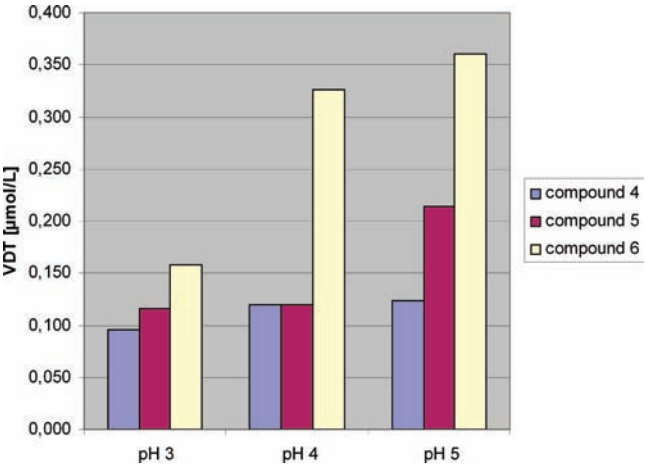


Figure 3. Visual detection thresholds (VDT) of the isolated acylated pigments at different pH conditions, calculated as micromoles per liter.

anthocyanins accompanied with an increasing pH stability of the pigment. When the visual strengths of the three acylated cyanidin 3-xylosyl(glucosyl)galactosides were compared with the thresholds of other nonacylated analogues previously determined by Stintzing et al.,[10] acylation resulted in a lower visual detection threshold combined with a higher strength of color. Figure 3 presents the data for the visual detection thresholds of the isolated acylated pigments calculated as micromoles per liter under different pH conditions.

Color Properties of Black Carrot Anthocyanin Solutions. In the CIELab color system, color is described by the parameters L^*, a^*, b^*, C^*, and h^*. L^* represents the lightness of the color (the lower the value, the darker the color), C^* is the chromaticity (chroma), a^* describes the red/green balance of color ($a^* > 0$ red, $a^* < 0$ green), and b^* describes the yellow/blue part ($b^* > 0$ yellow, $b^* < 0$ blue). These coordinates give access to new indices, the hue angle h^* [h_{ab} = arctan (b^*/a^*)], which represents the basic color.[25] A red color has h_{ab} around 0° (360°), yellow is described by h_{ab} around 90°, green has h_{ab} around 180° (−180°), while blue colors have h_{ab} values around 270° (−90°). By using hue angles, it is possible to classify the different black carrot samples according to colors (Table 4). At the highest anthocyanin concentration (45 mg/100 mL), the dark varieties Antonina and Deep Purple (L^* = 30.1 and 30.9) exhibited relative small coordinates, indicating an important blueness (b^* = −13.64 and −14.44) and a hue angle close to 340°. Increasing anthocyanin concentration slightly increased the hue angle (color) of the two black carrots from red (h_{ab} = 12.15° and 10.31°) toward more red-purple color (h_{ab} = 344.8° and 343.5°, respectively). Thus, these cultivars characterized by the darkest color contained also the largest anthocyanin amount (17.7 and 12.5 mg/100 g fw). Greater changes in hue angle with increasing anthocyanin concentration were also observed for the varieties Purple Haze and Beta Sweet; a total anthocyanin amount ranging from 1.5 to 8.1 mg/100 g fw, associated with a strong positive coordinate of axis a^* (redness) led to an increase in hue angle, a hue angle between 0° (360°) and 15° characterizing a reddish shade.[25] The presence of different and additional acylating groups might be responsible for color differences in the four anthocyanin extracts. From a chemical viewpoint, these four cultivars were all characterized by anthocyanins dominated by cyanidin glycosides. Accumulation of cyanidin derivatives give a wide color spectrum characterized by hue angle differences of about 25° between the most bluish (h_{ab} = 344.8°) and a red color (h_{ab} = 12°).

Table 4. Color Characteristics (CIELab) of Black Carrot Varieties at pH 3.5

concn (mg/100 mL)	C^*	h_{ab}	L^*	a^*	b^*
		Antonina			
15	35.92	12.15	63.7	35.12	7.56
30	47.06	359.33	52.4	46.53	−0.37
45	51.6	344.8	30.1	49.35	−13.64
		Deep Purple			
15	36.49	10.31	65.3	35.90	6.53
30	46.35	359.25	52.9	46.34	−0.54
45	49.3	343.5	30.9	47.33	−14.44
		Beta Sweet			
15	47.57	359.29	64.5	47.57	−0.59
30	63.53	6.21	51.1	63.16	6.87
45	68.35	11.68	43.3	66.93	13.84
		Purple Haze			
15	32.34	357.70	71.8	32.31	−1.30
30	49.07	1.65	58.6	49.05	1.41
45	61.49	7.64	45.5	60.94	8.17

The color activity concept and the CIELab color system are useful tools for study of key colorants in complex mixtures and help to understand the contribution of individual pigments to the overall color of carrots. Measurement of anthocyanin content is, in general, important for research as well as for industrial applications. However, learning more about their chemical properties may help to better understand the principles influencing anthocyanin bioavailability, tissue retention, and mechanisms of antioxidant action. HSCCC allows the isolation of pure anthocyanin standards in high amounts that were previously commercially unavailable, for further examinations on their biological properties.

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