Anthocyanins, Colour and Antioxidant Properties of Eggplant (*Solanum melongena* L.) and Violet Pepper (*Capsicum annuum* L.) Peel Extracts

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Acetone extracts from eggplant (*Solanum melongena* L.) and violet pepper (*Capsicum annuum* L.) peels both belonging to the Solanaceae plant family were characterized with respect to their anthocyanin profiles, colour qualities and antioxidant capacities. According to HPLC-DAD-MS^3 analyses the major anthocyanin in eggplant was delphinidin-3-rutinoside, while the predominant pigment in violet pepper was assigned to delphinidin-3-*trans*-coumaroylrutinoside-5-glucoside. Since virtually all anthocyanins were delphinidin-based, the effect of acylation and glycosylation patterns on colour stability and antioxidant capacity could be assessed. Application of two *in vitro*-assays for antioxidant capacity assessment revealed that eggplant generally exhibited higher values compared to violet pepper which was ascribed to 3,5-diglycosylated structures predominating in the latter. The higher extent of acylation in violet pepper was reflected by a more purplish colour shade of the extracts, but did not translate into a higher stability against fading which again was attributed to additional glycosyl substitution at C5. These findings support the relevance of structure-related activities of anthocyanins both for understanding food colour and their particular nutritional value.

Key words: Eggplant, Violet Pepper, Anthocyanin

Introduction

Eggplant (*Solanum melongena* L., Solanaceae) originating from Asia is one of the most widespread vegetables in the world. There is a big diversity of eggplant cultivars on the market varying in shape and colour, the most common ones being dark purple or violet. This colour is due to anthocyanins widely distributed in fruits, vegetables and grains (Mazza and Miniati, 1993), but also flowers and grasses (Fossen *et al*., 2002; Haslam, 1995). The most common eggplant anthocyanin is nasunin (delphinidin-3-*p*-coumaroylrutinoside-5-glucoside), the presence of which was first reported by Kuroda and Wada (cited in Mazza and Miniati, 1993). Nasunin occurs as *cis*- and *trans*-isomers, and the latter has been reported to be more stable. In contrast, only delphinidin-3-rutinoside and small quantities of delphinidin-3-rutinoside-5-glucoside were found in Bulgarian eggplant (Tanchev *et al.*, 1970), being devoid of acylglycosides. Thus, the presence or absence of acylation was assumed to be due to genetic differences which would allow to differentiate fruits from different provenances. Among the sugar substituents rhamnose and glucose were reported, while *p*-coumaric, caffeic and ferulic acids constituted the typical acyl moieties (Mazza and Miniati, 1993).

Violet pepper (*Capsicum annuum* L., Solanaceae) belonging to the same plant family like eggplant is a typical Mediterranean plant preferring warm climates. Pepper colour is usually due to a complex mixture of carotenoids such as capsanthin, capsorubin, cryptoxanthin, lutein and zeaxanthin esters but also chlorophylls (Schweiggert *et al*., 2005). Therefore, it is rather rare that the fruit contains anthocyanins. However, mutual co-occurrence of anthocyanins and carotenoids predominating in Solanaceae fruits is also known from tamarillo [*Cyphomandra betaceace* (Cav.) Sendt.] (Wrolstad and Heatherbell, 1974) and tomato [*Lycopersicon esculentum* (L.) Mill.] (Jones *et al*., 2003). Sharma and Seshardi (cited in Mazza and Miniati, 1993) reported the presence of an unusual anthocyanin (petunidin diglucoside) in four cultivars of chilli peppers grown in India. Saga and

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Kikuchi (cited in Mazza and Miniati, 1993) characterized nasunin in *C. annuum Goshiki*, the purple ripening stage being a particular character of this variety. However, no further data on the anthocyanin pattern of pepper have yet been reported. Much attention is also paid to the bioactivity of natural antioxidants in fruits and vegetables, because these components may reduce the level of oxidative stress (Scalbert *et al.*, 2005). On the other hand, colour stability at different pH values is one of the points of interest for the food industry (Stintzing and Carle, 2004). Regarding both aspects, the anthocyanin patterns of eggplant and violet pepper peels were investigated and related to their respective appearance at pH 1, pH 3.5 and pH 6. Moreover, the *in vitro* antioxidant capacities of peel extracts were assessed to establish structure-activity relationships.

Materials and Methods

Solvents and reagents

Reagents and solvents were purchased from VWR International (Darmstadt, Germany) and were of analytical or HPLC grade. Deionized water was used throughout.

Plant material and extraction

Violet pepper and eggplant were obtained from a local market in Stuttgart, Germany. The peel was manually removed and immediately frozen in liquid nitrogen, comminuted to obtain a fine powder and stored frozen at −80 °C until analyses. Exactly 5 g of eggplant and 1.5 g of pepper were extracted with aqueous acetone (pH 1, water acidified with trifluoroacetic acid/acetone, 30:70, v/v) at a ratio of 1 part solid sample and 2 parts extraction medium. After extraction under continuous stirring for 60 min, the solution was passed through a Buchner funnel with a filter paper (Schleicher & Schuell, Dassel, Germany). The obtained filtrate was concentrated *in vacuo* at 28 °C to dryness. Eggplant and pepper samples were re-dissolved in 5 mL and 2 mL purified water, respectively. The resulting extracts were used for determination of the total anthocyanin content, for colour assessment and pigment identification.

Anthocyanin content and colour measurements

Total anthocyanin content determination was based on a pH-differential method and expressed as delphinidin-3-glucoside equivalents (Giusti and Wrolstad, 2005) according to the following formula: c [mg/L] = $A \cdot M \cdot \mathrm{DF}/\varepsilon_M \cdot d$, with A = absorption value, M = molecular weight of delphinidin-3-glucoside (465 g/mol), DF = dilution factor, ε_M = molar extinction coefficient of delphinidin-3-glucoside at pH 1 (29000 L/mol·cm), and d = pathlength of the cuvette (1 cm). The relative peak area ratios of the chromatogram at 520 nm were registered after their identification by HPLC-DAD-MS^3 measurements (see below). All samples were analyzed in duplicate by a UV-vis spectrophotometer (Perkin Elmer, Überlingen, Germany) equipped with UV-vis (UV-Winlab version 2.85.04) and colour (Wincol version 2.05) softwares (Perkin Elmer, Norwalk, CT, USA). Based on absorption measurements covering the range from 380 to 780 nm, objective colour measurements (CIEL*a*b*) were carried out after 30 min of equilibration in McIlvaine buffer solutions at pH 6 and pH 3.5 as well as in Clark-Lubbs buffer at pH 1, respectively. Chromaticity C* [$C^* = (a^{*2} + b^{*2})^{1/2}$] und hue angle h° [h° = arctan(b*/a*)] were calculated (Gonnet, 1999) from a*- and b*-values at D_{65} and an observer angle of 10°. Also colour differences $\Delta E^* = [(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2]^{1/2}$ were calculated between pH 1 (pH value of highest anthocyanin stability) and pH 3.5 (typical pH value of food, intermediate anthocyanin stability), as well as pH 1 and pH 6 (low anthocyanin stability), respectively.

Anthocyanin pattern characterization by HPLC-DAD and LC-MS^3

Using a Merck LaChrom Elite HPLC System (Merck-Hitachi, Darmstadt, Germany) equipped with an autosampler L-2200, a pump L-2130, a diode array detector L-2450, and a JetStream column oven, anthocyanins were separated on an analytical C18 Sunfire column (250 × 4.6 mm, 5 μm; Waters, Wexford, Ireland) with a C18 pre-column (4 × 3.0 mm i.d., Phenomenex, Torrance, CA, USA) at a constant temperature of 25 °C and a flow rate of 1 mL/min. Eluent A was 5% aqueous formic acid, 100% acetonitrile was used as eluent B. Starting isocratically with 100% A for 5 min, linear gradients were followed to 10% B at 20 min, 13% B at 40 min, 20% B at 44 min, 25% B at 50 min, and finally 100% B at 55 min before re-equilibration to initial conditions. Monitoring was performed at 520 nm.

Using the same method, mass spectrometric detection was carried out on an Agilent Series 1100 HPLC system (Agilent, Waldbronn, Germany) interfaced with a Bruker Model Esquire 3000+ ion trap mass spectrometer (Bruker, Bremen, Germany) operating in the positive ionisation mode.

Antioxidant capacity

Acetone water extracts were adjusted to an absorption of $A = 1.00 \pm 0.05$ equivalent to a comparable anthocyanin concentration using a pH 1 buffer. The same extract-solvent-ratio was subsequently applied for dilution with deionized water prior to further dilution with the specific buffers for antioxidant capacity measurements. The TEAC (Trolox equivalent antioxidant capacity, Trolox = 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid; Van den Berg *et al.*, 1999) and FRAP (ferric reducing capacity antioxidant power; Benzie and Strain, 1996) assays were applied. For calibration purposes, the water-soluble vitamin E analogue Trolox (Trolox equivalents) as well as L-ascorbic acid (vitamin C equivalents) were used according to Kim and Lee (2004). While the TEAC assay reflects the reducing capacity at neutral conditions by scavenging the $ABTS^{+}$ radical cation monitored at 734 nm, the FRAP assay is based on the reduction of Fe^{3+} to Fe^{2+} at acidic pH registered at 593 nm.

Results and Discussion

HPLC-DAD-MS^3 detection of anthocyanin

Figs. 1 and 2 show the typical HPLC chromatograms of violet pepper and eggplant peel extracts monitored at 520 nm. To assign the chemical structures of the individual anthocyanin peaks, LC-DAD-MS^3 measurements were performed and the results obtained were compared with literature data (Ando *et al.*, 1999; Ichiyanagi *et al.*, 2005; Wu and Prior, 2005). Four glycosylated structures were detected in eggplant. The major pigment was delphinidin-3-rutinoside (84%, **5**), followed by delphinidin-3-rutinoside-5-glucoside (8.1%, **1**), a delphinidin-derivative isobaric to **1** (4.2%, **2**) and delphinidin-3-glucoside (3.7%, **4**), respectively (Fig. 1, Table I). In contrast to Japanese eggplant (Ichiyanagi *et al.*, 2005), no acylglycosides were found. This observation conforms with previous findings by Tanchev *et al.* (1970) and Wu and Prior (2005) who reported the sole presence of anthocyanin glycosides in eggplant cultivars. In the present study, except for delphinidin-3-rutinosyl-glucoside (**2**), the same anthocyanins were detected in violet pepper. However, the major pigment from violet pepper was delphinidin-3-*trans*-coumaroylrutinoside-5-glucoside (89%, **9**) also known as nasunin, followed by delphinidin-3-*cis*-coumaroylrutinoside-5-glucoside (4.6%, **8**), delphinidin-3-caffeoylrutinoside-5-glucoside (2.4%, **7**), delphinidin-3-feruloylrutinoside-5-hexoside (1.2%, **10**), delphinidin-3-rutinoside (0.9%, **5**), delphinidin-3-glucoside (0.7%, **4**), delphinidin-3-rhamnoside (0.7%, **6**), and finally delphinidin-3-rutinoside-5-glucoside (0.5%, **1**). In addition, delphinidin-3-coumaroyl-hexoside (**12**) and petunidin-3-coumaroyl-hexoside (**13**) were identified (Table I, Fig. 2). *Cis*- and *trans*-isomers of nasunin were recently isolated by Ichiyanagi *et al.* (2005) from eggplant peel, but till

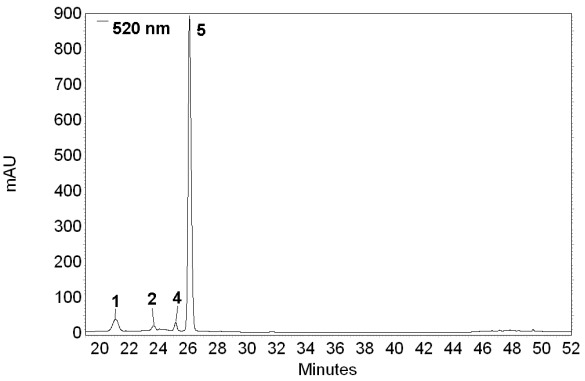

Fig. 1. HPLC-DAD chromatogram of a peel extract from eggplant monitored at 520 nm (peak assignment is given in Table I).

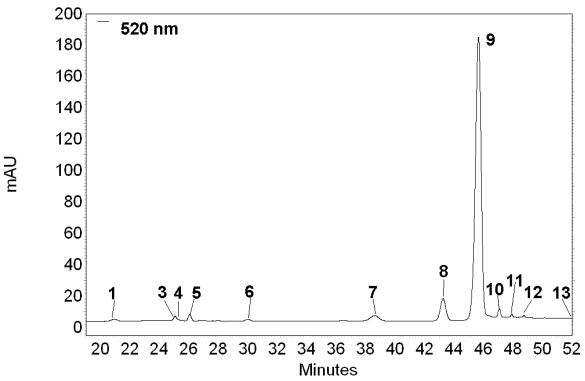

Fig. 2. HPLC-DAD chromatogram of a peel extract from violet pepper monitored at 520 nm (peak assignment is given in Table I).

Table I. HPLC-DAD-MS data and contents of anthocyanins from eggplant (EP) and violet pepper (VP) peel extracts (x = present).

Anthocyanin[a]		R_t [min]	$\lambda_{vis\text{-}max}$ [nm]	A_{310} / $A_{vis\text{-}max}$ (%)	*m/z* $[M]^+$	MS^2 *m/z* $[M]^+$	MS^3 *m/z* $[M]^+$	EP	VP	EP [mg/kg] ± SD[b]	VP [mg/kg] ± SD[b]
1	Del-3-rut-5-glc	20.8	526	–[c]	773	611, 465, 303	465, 303	x	x	36.5 ± 0.8	1.5 ± 0.1
2[d]	Del-3-rut-glc	23.3	522	–[c]	773	627, 465, 303	303	x		19.1 ± 0.1	
3	–[e]	24.3	526	–[c]	–[c]	–[c]	–[c]		x		–[c]
4	Del-3-glc	24.9	527	–[c]	465	303	303	x	x	16.5 ± 1.0	2.2 ± 0.0
5	Del-3-rut	25.8	525	–[c]	611	465, 303	303	x	x	378.0 ± 9.9	2.9 ± 0.2
6	Del-3-rham	30.0	526	–[c]	449	303	192		x		2.4 ± 0.5
7	Del-3-caffeoylrut-5-glc	38.5	529	51	935	773, 465, 303	611, 465, 303		x		7.8 ± 0.4
8	Del-3-*cis*-coumaroylrut-5-glc	43.2	532	52	919	757, 465, 303	611, 465, 303		x		14.7 ± 0.8
9	Del-3-*trans*-coumaroylrut-5-glc	45.8	530	80	919	757, 465, 303	611, 465, 303		x		286.2 ± 1.7
10[d]	Del-3-feruloylrut-5-hex	47.1	530	135	949	787, 465, 303	465, 303		x		3.8 ± 0.1
11	–[e]	48.0	525	–[c]	–[c]	–[c]	–[c]		x		–[c]
12[d]	Del-3-coumaroyl-hex	48.3	529	106	611	465, 303	303		x		–[c]
13[d]	Pet-3-coumaroyl-hex	51.9	–[c]	–[c]	625	479, 317	317		x		–[c]

[a] Del, delphinidin; Pet, petunidin; glc, glucoside; hex, hexoside; rham, rhamnoside; rut, rutinoside.
[b] Mean anthocyanin content ± standard deviation (n = 2).
[c] Not detected.
[d] Tentatively assigned.
[e] Not identified.

now have not been reported in violet pepper. Anthocyanins carrying hydroxycinnamoyl moieties have been found to predominantly occur in their *trans*-form in flower petals (Ando *et al.*, 1999; Yoshida *et al.*, 2003) and fruits (Inami *et al.*, 1996), respectively. On the other hand, *cis*-isomers exhibit molar absorption values about 1.5 to 2.4 times higher than their corresponding *trans*-isomers and have therefore been proposed as a tool to modify the level of colour expression and stability (George *et al.*, 2001; Inami *et al.*, 1996). Whereas irradiation may induce isomerisation from *trans*- into *cis*-isomers, absorption at 310 nm is of physiological relevance to prevent range UV-B irradiation thus exhibiting a cell protecting effect (Stintzing and Carle, 2004), which is missing in anthocyanin glycosides.

Interestingly, different fragmentation patterns for anthocyanins **1** and **2** yielding an identical molecular mass of 773 were found. The molecular cation $[M]^+$ = 773 of **1** was fragmented into *m/z* 611, 465 and 303. The loss of *m/z* 162 was due to anhydro-glucose split off at C5, followed by rhamnose (*m/z* 611 → 465) and finally glucose loss at C3 (*m/z* 465 → 303). In contrast, **2** was fragmented into *m/z* 627, 465 and 303. The first transition was ascribed to rhamnose (*m/z* 773 → 627), then glucose (*m/z* 627 → 465), and finally a second glucose (*m/z* 465 → 303) split off. Only minor signals for the 773 → 627 transfer were detected, indicating rare cleavage of a single rhamnose. In the case of delphinidin-3-rutinoside-5-glucoside (**1**), the elimination of glucose (*m/z* 773 → 611) produced an abundant signal, while this was not the case for **2**.

Whereas Wu and Prior (2005) tentatively identified two delphinidin derivatives with a molecular cation of $[M]^+ = 773$ in eggplant as delphinidin-3-rutinoside-5-galactoside and delphinidin-3-rutinoside-5-glucoside, respectively, the fragmentation pattern of **2** did not comply with any of these structures. In addition, **1** showed an $A_{440}/A_{vis\text{-}max}$ value of 0.23 compared to 0.46 for **2**, respectively. According to Santos-Buelga and Williamson (2003), 3,5-diglycosides will exhibit both lower $A_{440}/A_{vis\text{-}max}$-values and lower retention times than their corresponding 3-glycosides. Therefore, **1** and **2** were assigned to delphinidin-3-rutinoside-5-glucoside and delphinidin-3-rutinosyl-glucoside, respectively.

In compliance with data from previous reports (Ando *et al.*, 1999; Tatsuzawa *et al.*, 2000), compounds **4**, **5** and **6** were assigned to delphinidin-3-glucoside (*m/z* 465 → 303), delphinidin-3-rutinoside (*m/z* 611 → 465 → 303) and delphinidin-3-rhamnoside (*m/z* 449 → 303), respectively. The presence of delphinidin-3-galactoside as assumed for compound **3** was ruled out by coinjection of a blueberry juice extract containing this anthocyanin.

Anthocyanins **7** to **10** and **12** exhibited an additional absorption maximum at 310 nm pointing to hydroxycinnamoyl substituted structures. The $A_{310}/A_{vis\text{-}max}$-value reflecting the molar ratio of the particular acyl moieties and the anthocyanin was found to be higher for the *trans*- (**9**) than for the *cis*-isomer (**8**), thus confirming the findings by Tatsuzawa *et al.* (2000) and Ando *et al.* (1999) in *Petunia*. The molecular cation $[M]^+ = 919$ produced fragment ions at *m/z* 757, 611, 465 and 303. The considerably high abundance of *m/z* 757 compared to the other transitions *m/z* 757 → 611, 611 → 465, and 465 → 303 illustrates that glucose at C5 position is more readily cleaved (data not shown). The strong signal at $[M]^+ = 465$ indicates the loss of *p*-coumaroyl-rhamnose, while fragmentation between rhamnose and *p*-coumaric acid (*m/z* 611 → 465) was negligible. However, as recently reported (Ichiyanagi *et al.*, 2005), no differences in the fragmentation behaviour of *cis*- and *trans*-nasunin were registered. It is noteworthy that **8** being the galactosyl derivative of **9** could be excluded by the difference of the $A_{310}/A_{vis\text{-}max}$ ratio between **8** and **9** strongly supporting the presence of *cis*- and *trans*-isomers.

Pigment **7** yielded a molecular cation at $[M]^+ = 935$. According to its fragmentation into *m/z* 773, 611, 465, and 303, and its retention in a reversed-phase system, compound **7** was assigned to delphinidin-3-caffeoylrutinoside-5-glucoside. The first transition of *m/z* 162 was ascribed to glucose which was the most common fragmentation product compared to *m/z* 773 → 611 (caffeic acid), 611 → 465 (rhamnose) and 465 → 303 (glucose), respectively. Compound **10** with a molecular ion at $[M]^+ = 949$ and the specific mass defects of *m/z* 162 (*m/z* 949 → 787) and *m/z* 322 (*m/z* 787 → 465, feruloylrhamnose) was assigned to delphinidin-3-feruloylrutinoside-5-hexoside. This conforms with previous reports where feruloyl acylation brought about a decrease in polarity as compared to caffeoyl- or coumaroyl moieties (Santos-Buelga and Williamson, 2003). Compound **12** represents the acylated monoglucoside delphinidin-3-coumaroyl-hexoside with a higher retention time than the corresponding triglycoside **9**. According to the specific transitions upon fragmentation yielding an aglycon of *m/z* 317, **13** was tentatively assigned to petunidin-3-coumaroyl-hexoside. It is anticipated that the hexoside moiety of **10**, **12** and **13** is glucose rather than galactose, because the latter sugar has scarcely been reported to constitute anthocyanins from the Solanaceae.

Colour quality and stability

It is well established that anthocyanins are unstable and readily fade at pH values greater than 2 (Stintzing and Carle, 2004). In comparison to eggplant, the absorption maximum of violet pepper extracts was bathochromically shifted by 12 nm to 532.8 nm. This colour change can be ascribed to the presence of acylated anthocyanins. The monomeric anthocyanin content as determined by a pH differential method (Giusti and Wrolstad, 2005), exhibited a greater anthocyanin content of 450.1 mg/kg fresh weight for eggplant compared to violet pepper yielding 321.5 mg/kg fresh weight, the former being in the range reported for red raspberries and red radishes, respectively (Giusti and Wrolstad, 2005; Wang *et al.*, 1997). The deeply purple appearance of eggplant and pepper is due to delphinidin-type pigments, modified by pigment acylation and total anthocyanin concentration. A third colour modulating principle was reported by Nothmann *et al.* (1976) who ascribed a minor darkening effect to the presence of chlorophylls. Colour properties at different pH values after 30 min equilibration are shown

Table II. Colour properties of eggplant and violet pepper peel extracts at different pH values on the same tinctorial strength of $A = 1.00 \pm 0.05$ at pH 1.

		Eggplant (± SD)[a]	Violet pepper (± SD)[a]
pH 1	L*	65.83 ± 0.32	62.97 ± 0.21
	C*	52.12 ± 0.56	61.60 ± 0.33
	h°	8.92 ± 0.20	340.99 ± 0.04
pH 3.5	L*	80.56 ± 0.46	79.56 ± 0.87
	C*	23.46 ± 0.75	25.18 ± 0.63
	h°	19.50 ± 1.99	336.2 ± 0.11
pH 6	L*	83.44 ± 0.93	87.52 ± 1.49
	C*	8.83 ± 0.30	5.44 ± 1.87
	h°	60.94 ± 1.08	325.81 ± 6.20
ΔE* (pH 1–pH 3.5)		32.86	40.15
ΔE* (pH 1–pH 6)		50.38	61.43
CS value[b] (pH 1–pH 3.5)		0.37	0.36
CS value[b] (pH 1–pH 6)		0.21	0.16

[a] SD, standard deviation ($n = 4$).
[b] CS value, colour stability A(pH 6)/A(pH 1); A(pH 3.5)/A(pH 1).

in Table II. It is quite obvious that the particular pigment patterns affect colour stability. Anthocyanins are most stable in their flavylium cation form at acidic pH (pH $\leq$ 2), losing colour at pH 3–4 through hemiketal formation further being transformed into quinoidal bases (Stintzing and Carle, 2004). Cabrita *et al.* (2000) observed an absorption decrease of anthocyanin-3-glucosides until pH 6 being increased at alkaline pH. To get a clearer insight into eggplant and violet pepper appearance as affected by pH increments, colour stability values according to Dougall *et al.* (1997) were calculated (Table II), with higher numbers translating into improved pigment retention. These ratios were lower for violet pepper than for eggplant, especially between pH 1 and pH 6 (Table II). ΔE* values (Gonnet, 1999) were also measured to monitor colour differences which were generally higher for violet pepper (Table II). These findings demonstrate that ΔE* values present a more sensitive tool to assess colour alterations than CS values, because the latter exclusively consider absorptivity values. Hence it follows that despite acylation pigment extracts from violet pepper exhibited a lower tinctorial strength. Inspection of individual chromatic data revealed that lightness L* increased in less acidic solutions being more intense between pH 1 and pH 3.5 complying with the CS values (Table II). Hue angle values for eggplant were located in the red region and reached 60.9 with increasing pH value, equivalent to an orange-yellow tint. In contrast, violet pepper exhibited a purplish-blue colour shifting towards bluish tones upon neutralisation (Table II). Hayashi *et al.* (1996) have reported Hunter h values of 341 for eggplant at pH 3.16. This difference may be due to the absence of acylated anthocyanins in eggplant samples in the present work. Chroma was higher in violet pepper (61.6) than in eggplant (52.1) and decreased in both samples with increasing pH value being faster for violet pepper than for eggplant, respectively (Table II). This was unexpected because acylation was reported to enhance pigment stability and thus colour brilliance C* (Giusti and Wrolstad, 2003; Redus *et al.*, 1999; Stintzing *et al.*, 2002a). Accordingly, eggplant anthocyanins should be more prone to colour fading. However, sugar substitution at the C5 position was earlier shown to decrease the hydration constant pK_H by rendering the rotation of aromatic acyl moieties to fold over the flavylium nucleus impossible (Stintzing *et al.*, 2002a). Thus, enhanced proneness of 3,5-diglycoside derivatives towards fading is plausible.

In vitro antioxidant capacity of eggplant and violet pepper anthocyanins

The ability of anthocyanins to scavenge free radicals has been primarily related to the catechol function of the B-ring (Kim and Lee, 2004; Rice-Evans *et al.*, 1996), but may also be dependent on type and extent of glycosylation and acylation, respectively (Stintzing *et al.*, 2002a; Wang *et al.*, 1997). The most common acylating moieties include cinnamic, caffeic, *p*-coumaric, ferulic and sinapic acids, the first three occurring in violet pepper (Table I). Contrary to eggplant being devoid of acylated structures, violet pepper exhibited 97% acylated anthocyanins.

To get an insight into structurally related antioxidant capacities, acetone extracts of eggplant and violet pepper were measured by TEAC and FRAP assays based on the same anthocyanin content. Although eggplant was devoid of acylated anthocyanins, its extracts exhibited higher antioxidant values than those from violet pepper (Table III). This appears to be in contrast to findings of Stintzing *et al.* (2002a) demonstrating that isolated acylglycosides exhibited higher ORAC (oxygen radical absorbing capacity) values than their corresponding glycosides. On the other hand, it was also shown that whole pigment extracts from black-

Table III. *In vitro* antioxidant capacity of eggplant and violet pepper extracts on the same quantitative anthocyanin basis of 17 mg/L determined by TEAC and FRAP assays expressed as vitamin C and Trolox equivalents [mg/L], respectively.[a]

	Eggplant	Violet pepper
TEAC (Trolox equivalent)	217.9 ± 12.8	196.1 ± 10.3
TEAC (vitamin C equivalent)	132.2 ± 7.5	119.3 ± 6.1
FRAP (Trolox equivalent)	132.7 ± 2.4	118.2 ± 5.2
FRAP (vitamin C equivalent)	91.1 ± 1.7	80.8 ± 3.7

[a] Mean ± standard deviation (n = 4).

berry being devoid of acylated structures afforded higher antioxidant values than those from black carrot and red cabbage that contain a high percentage of acylated structures (Stintzing *et al.*, 2002a). In another study (Stintzing *et al.*, 2002b) when the FRAP values of various anthocyanin isolates were assessed, pigments substituted with sugars both at the C3 and C5 position exhibited a lower antioxidant capacity than their corresponding anthocyanin-3-glycosides. In the present work, the same appeared to be valid for eggplant with 84% of the anthocyanin fraction being delphinidin-3-rutinoside and a higher antioxidant activity compared to violet pepper with 93.6% delphinidin-3-coumaroylrutinoside-5-glucoside. Related studies with purified anthocyanins and extracts have shown that delphinidin-3-rutinoside with 3.7 μmol Trolox/mg afforded higher values than sinapic acid derivatives of cyanidin-3-diglucoside-5-monoglucoside affording 2.6 μmol Trolox/mg, respectively (Degenhardt *et al.*, 2000). The findings of the present work also corroborate those by Nakajima *et al.* (2003) who found that radical scavenging potential of anthocyanins and flavonols against radical cations may be either retained or decreased by the introduction of an acyl moiety.

Previously, the antioxidant activity of anthocyanins was chiefly related to the aglycon structure (Rice-Evans *et al.*, 1996; Wang *et al.*, 1997). Wang *et al.* (1999) showed that the smaller the number of sugar units at C3, the higher the antioxidant activity with cyanidin being more active than its glucoside. Hence, the number of sugar residues at the C3 and C5 position seems to be crucial for antioxidant activity. Moreover, different sugars may have varying effects on the antioxidant activity. For example, 3-substituted anthocyanins exhibited higher ORAC activity for glucose and rhamnose substitutions, but decreasing activity for galactose substituents (Wang *et al.*, 1997). Hydroxylation at the B-ring will increase antioxidant capacity, *i.e.* delphinidin had a higher antioxidative activity than cyanidin, but hydroxy- or methoxy-substitution at the positions 3, 5, and 7 of rings A and C were less important (Rice-Evans *et al.*, 1996).

These findings together with the results of the present work strongly support the concept of antioxidant power modulation by different substitution patterns of anthocyanins. FRAP values were always lower than those from TEAC assays on the particular equivalent basis, while Trolox-based figures exceeded vitamin C equivalent numbers in the test system (Table III). It is further noteworthy that TEAC and FRAP assays are not only based on varying mechanisms but apply different pH and solvent systems, affecting the radical capacity values of the respective sample. While the TEAC test is carried out at pH 7.4 typical for physiological conditions, the FRAP assay is conducted at pH 3.6 mainly relevant for food. This should be considered for data evaluation and comparison with literature data.

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