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Evaluation of the antioxidant capacity of betalainic fruits and vegetables

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Summary

The present investigation determined total phenolics, ascorbic acid, betalain contents and the corresponding antioxidant capacities of betalain-bearing fruits and vegetables. In addition to differently coloured Swiss chard petioles (*Beta vulgaris* L. ssp. *cicla* [L.] Alef. cv. 'Bright Lights') and hypocotyls of white, yellow, and red beetroot varieties (*Beta vulgaris* L. ssp. *vulgaris*, cv. 'Albina Vereduna', cv. 'Burpee's Golden', and cv. 'Rote Kugel 2'), juices from cactus pears (*Opuntia ficus-indica* [L.] Mill. cv. 'Gialla' and cv. 'Rossa') and pitaya fruits (*Hylocereus polyrhizus* [Weber] Britton & Rose, *H. undatus* [Haworth] Britton & Rose, *Selenicereus megalanthus* [K. Schumann ex Vaupel] Moran) were included in this study. Antioxidant capacities were determined by application of the TEAC and FRAP assays, respectively, resulting in differing rankings of the commodities investigated. In both test systems, highest antioxidant capacity was shown for red beetroot extract while for the remaining samples no straightforward order could be established.

Introduction

Chlorophylls, carotenoids, anthocyanins, and betalains represent the four most important pigment classes imparting all kinds of colour shades to fruit and vegetables. According to current conception, the same pigments are considered to promote human health and well-being. However, scarce information is available on betalainic food crops, although knowledge on their bioactivity would allow to judge their nutritional quality more objectively. Betalainic fruits and vegetables are consumed fresh or after cooking and may thus contribute to healthy nutrition. In addition, highly processed products to which betalain-containing colour preparations are added may be improved not only visually but also from a nutritional point of view. In this respect, both betaxanthins (yellow-orange betalains, Fig. 1) and betacyanins (red-violet betalains, Fig. 2) have recently attracted attention for their antioxidative activities which were proven to surpass even the values of classical dietary antioxidants such as ascorbic acid, rutin, and catechin (CAI et al., 2003). The antioxidant properties of betacyanins are ascribed to their free phenolic group(s). Additionally, the cyclic amine group of the betalamic acid moiety resembling the structure of the very strong antioxidant ethoxyquin is considered to act as hydrogen donor both in betacyanins and betaxanthins (KANNER et al., 2001). Other studies attributed the antioxidant potential of betalain-containing foodstuffs mainly to their polyphenolic compounds without considering the role of betalains (GALATI et al., 2003; 2005; KUTI, 2004; PYO et al., 2004). Hence, a range of differently coloured Swiss chard petioles, beetroots and cactus fruits were selected to assess their potential antioxidant capacity applying the most widespread TEAC (Trolox equivalent antioxidant capacity) and FRAP (ferric ion reducing antioxidant power) assays. The unpigmented representatives of the respective commodities were also included to single out the effect of the betalains. Major antioxidant compounds, namely total phenolics, ascorbic acid, and betalains were determined to scrutinise their particular contribution to the total antioxidant capacity of the food crops investigated.

Materials and methods

Plant material

Differently coloured Swiss chard (*Beta vulgaris* L. ssp. *cicla* [L.] Alef. cv. 'Bright Lights'; Chenopodiaceae; LANGE et al., 1999) exhibiting white, yellow, orange and red-purple petioles as well as different

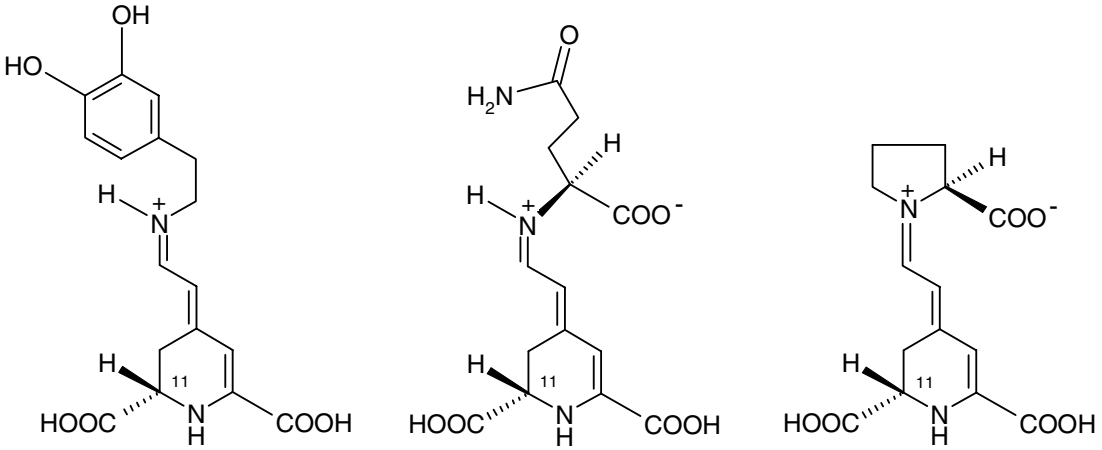

Fig. 1: Structures of dopamine-bx (= miraxanthin V; major betaxanthin in yellow Swiss chard stems), glutamine-bx (= vulgaxanthin I; major betaxanthin in yellow and red beetroots), and proline-bx (= indicaxanthin; major betaxanthin in betalainic cactus pears).

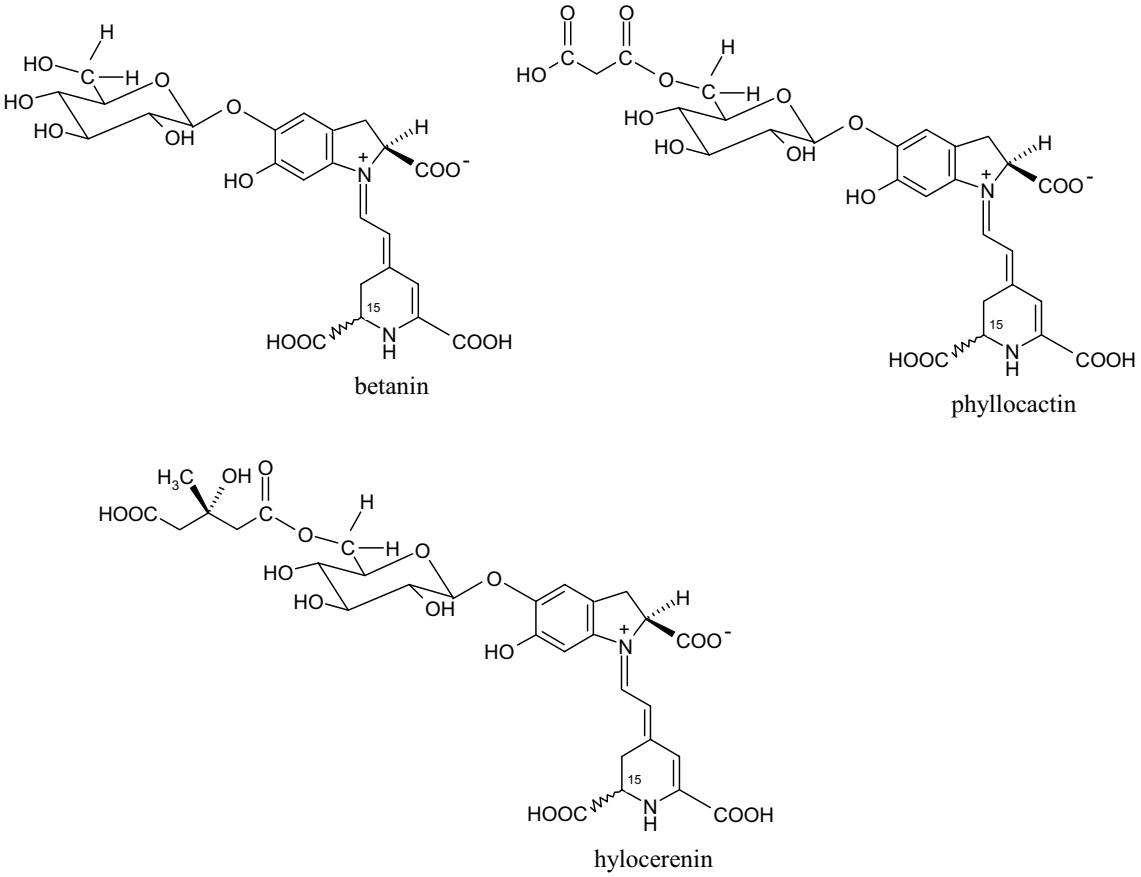

Fig. 2: Structures of betanin (major betacyanin in red beetroot, red-purple Swiss chard stems, and betalainic cactus pears), phyllocactin and hylocerenin (the latter both representing major betacyanins in fruits from *H. polyrhizus*).

beetroot cultivars (*Beta vulgaris* L. ssp. *vulgaris* cv. 'Albina Vereduna', cv. 'Burpee's Golden', and cv. 'Rote Kugel 2'; Chenopodiaceae; LANGE et al., 1999) developing white, yellow, and red coloured hypocotyls, respectively, were harvested at the end of September 2005 on the Experimental Station for Horticulture at Hohenheim University. After washing the Swiss chard plants, leaves were removed from the petioles and the latter immediately frozen in liquid nitrogen, sealed in plastic bags under reduced pressure, and stored at -80°C until further processing. Only fully developed hypocotyls showing regular pigment distribution were selected and investigated in this study. After washing, beetroots were deprived of their hair roots, cut in quarters by longitudinal section and frozen in liquid nitrogen and subsequently treated as described for Swiss chard petioles.

Cactus pear and pitaya fruits were purchased at the beginning of November 2005 on a local market, stored at 4°C, and processed within one week to prevent microbial spoilage. Cactus pears (*Opuntia ficus-indica* [L.] Mill. cv. 'Gialla' and cv. 'Rossa'; Cactaceae) were from Sicily (Italy). Pitaya fruits from *Hylocereus polyrhizus* [Weber] Britton & Rose (Cactaceae) with purple pulp were obtained from Israel, whereas fruits from *H. undatus* [Haworth] Britton & Rose (Cactaceae) with white pulp were from Vietnam, and fruits from the *Selenicereus megalanthus* [K. Schumann ex Vaupel] Moran (Cactaceae) with yellow peel and white flesh from Ecuador, respectively.

Solvents and reagents

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

Cryogenic milling of Swiss chard petioles and beetroot hypocotyls

As described earlier (KUGLER et al., 2004), comminution of Swiss chard petioles and beetroot hypocotyls was performed in a Waring blender model 38BL41 (Waring Products, Torrington, CT, USA). To prevent enzymatic reactions during grinding, liquid nitrogen was added. The resulting powder was directly extracted as described below.

Extraction of Swiss chard and beetroot powder

Exactly 50.000 g of Swiss chard and beetroot powder, respectively, were extracted by addition of 200 mL of 60% aqueous methanol (v/v) to inhibit residual enzyme activity (KUGLER et al., 2004). After stirring for 30 min at room temperature, the plant material was separated from the extract by filtering through a glass frit under reduced pressure and rinsing with 100 mL extraction solvent. The resulting extracts were concentrated *in vacuo* at 30°C and diluted to a volume of 50 mL with purified water after temperature adjustment for 15 min at 20°C in a water bath. Finally, the aqueous extracts were membrane-filtered (0.45 µm; Acrodisc Premium Filter, Pall, Ann Arbor, MI, USA) before storage at -80°C until analysis.

Extraction of cactus pears and pitaya fruits

Cactus pears and pitaya fruits were cut in halves, and the pulp was separated from the peel before manually squeezing of the former. The obtained juice was clarified by paper filtration (Macherey & Nagel, Düren, Germany) and immediately stored at -80°C. Before analysis, juice samples were thawed and membrane-filtered (0.45 µm).

Isolation and purification of betanin from red beet

Betanin was isolated and purified according to a previously applied procedure (STINTZING et al., 2004).

Photometric determination of total phenolics

Total phenolics were assessed by the Folin-Ciocalteu assay (SINGLETON and ROSSI, 1965): A volume of 100 µL of sample extract or juice, respectively, was diluted to 1000 µL with purified water in a test tube prior to addition of 100 µL Folin-Ciocalteu reagent. For red beetroot extract, dilution to a volume of 2000 µL was required. After vortexing, the reaction was allowed to proceed for 3 min before adding 800 µL of sodium carbonate (7.5%). After repeated vortexing, the sample was allowed to stand for 60 min. Absorption at 720 nm was measured in a UV-vis spectrometer (Perkin-Elmer, Überlingen, Germany) equipped with UVWinLab V 2.85.04 software (Perkin-Elmer Instruments, Norwalk, CT, USA). All determinations were performed in duplicate. Based on a four point calibration, total phenolic contents were expressed as gallic acid (GAE) or ascorbic acid equivalents (AAE), respectively.

Determination of ascorbic acid

Ascorbic acid contents of appropriately diluted sample extracts and juices, respectively, were determined in duplicate using an enzyme test kit (R-Biopharm, Darmstadt, Germany) based on the selective oxidation of ascorbic acid to dehydroascorbic acid and registering formazan formation at 578 nm.

Photometric quantification of betalains

The aqueous pigment extracts were diluted with McIlvaine buffer (pH 6.0, citrate-phosphate) to obtain absorption values of $0.9 \leq A \leq 1.1$ at their respective absorption maxima. Measurements were performed in triplicate and the betalain content (BC) was calculated according to STINTZING et al. (2003): BC [mg/L] = [(A*DF*MW*1000)/(ε*l)] where A is the absorption value at the absorption maximum corrected by the absorption at 650 nm, DF the dilution factor and l the path-length (1 cm) of the cuvette. For quantification of betacyanins, the molecular weight (MW) and molar extinction coefficient (ε) of betanin [MW = 550 g/mol; ε = 60,000 L/(mol*cm) in H_2O; KUGLER et al., 2004] at their respective absorption maximum were applied. Quantitative equivalents of the major betaxanthins (bx) were determined at their respective absorption maximum applying a mean molar extinction coefficient [ε = 48,000 L/(mol*cm) in H_2O; KUGLER et al., 2004] and the molecular weight of glutamine-bx (vulgaxanthin I; MW = 339 g/mol) for the extracts from differently coloured Swiss chard and beetroots (KUGLER et al., 2004; STINTZING et al., 2002) and of proline-bx (indicaxanthin; MW = 308 g/mol) for coloured juices from cactus pear fruits (STINTZING et al., 2002). Determinations were performed with a UV-vis spectrometer (Perkin-Elmer, Überlingen, Germany) equipped with UVWinLab V 2.85.04 software (Perkin-Elmer Instruments, Norwalk, CT, USA).

Antioxidant capacity measurements

TEAC assay. Based on an improved TEAC assay (VAN DEN BERG et al., 2000), radical anions of ABTS [2,2´-azinobis-(3-ethylbenzthiazoline)-6-sulfonate] were obtained by oxidation of the latter with peroxyl free radicals generated by thermal decomposition of ABAP [2,2´-azobis-(2-amidinopropane)-dihydrochloride]. The diammonium salt of ABTS was dissolved in a pH 7.4 phosphate-buffer medium (Na_2HPO_4/KH_2PO_4 containing NaCl) at a concentration of 20 mmol/L. A volume of 0.5 mL of this radical precursor solution was added to 100 mL of a solution of ABAP in phosphate-buffer (2.5 mmol/L) and incubated for 15 min at 60°C in the dark. To an aliquot of 2000 µL of the ABTS radical solution, 40 µL of appropriately diluted sample extract or juice, respectively, were added and mixed thoroughly. After a reaction time of exactly 6 min, absorbance was registered photometrically at 734 nm. All determinations were performed in duplicate, and for each duplicate determination a freshly pipetted blank (2000 µL ABTS radical solution + 40 µL of purified water) was measured.
Standard solutions of the vitamin E analogue Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid), ascorbic acid, and dopamine hydrochloride were used for calibration. Antioxidant capacities were expressed as equivalents of Trolox (TE), ascorbic acid (AAE), and dopamine (DAE), respectively.

FRAP assay. The ferric ion reducing antioxidant power assay, which is based on the reduction of the Fe^{3+} complex of 2,4,6-tripyridyl-*s*-triazine [$Fe(TPTZ)^{3+}$] to the intensely blue coloured corresponding Fe^{2+} complex [$Fe(TPTZ)^{2+}$], was performed as described earlier (BENZIE and STRAIN, 1996; GARDNER et al., 2000). The FRAP reagent was obtained by mixing 25 mL of sodium acetate-buffer medium (pH 3.6) with 2.5 mL from a solution of TPTZ dissolved in diluted hydrochloric acid, and a volume of 2.5 mL from an aqueous solution of $FeCl_3$. To an aliquot of appropriately diluted sample extract or juice, respectively, 1500 µL of the FRAP reagent were added. Following thorough mixing, absorbance was registered at 593 nm after a reaction time of exactly 4 min at room temperature. All measurements were performed in duplicate.
Standard solutions of the vitamin E analogue Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid), of ascorbic acid, and of dopamine hydrochloride were used for calibration. Antioxidant capacities were expressed as Trolox (TE), ascorbic acid (AAE), and dopamine equivalents (DAE), respectively.

In addition, the antioxidant activity of purified betanin was assessed in each assay based on a five-point-calibration ($R^2 > 0.997$) to single out the particular contribution of betacyanins.

Results and discussion

Chenopodiaceae

White, yellow, orange, and red-purple coloured Swiss chard stems from the cultivar 'Bright Lights' earlier characterised (KUGLER et al., 2004; 2006), as well as red, yellow and white beetroot phenotypes were included in this study to investigate the impact of betalains on their overall antioxidant capacity. Moreover, total phenolics, ascorbic acid, and betalain contents were assessed for correlation with TEAC and FRAP data. According to the suggestion by KIM et al. (2002), concentration of total phenolics and antioxidant capacities were also calculated as ascorbic acid equivalents (AAE) to facilitate comparison with well-established antioxidants (see Tab. 1).
The highest total phenolics value (140.7 mg GAE/100 g fresh weight) was determined for the red beetroot extract, whereas red-purple coloured Swiss chard petioles exhibited the lowest total phenolics concentration (31.2 mg GAE/100 g fresh weight) of all investigated Chenopodiaceae representatives. In beetroot tubers, WINTER and HERRMANN (1986) detected conjugates of caffeic, *p*-coumaric, as well as ferulic acids depending on the cultivar. In another study on phenolic compounds of red beetroot, the presence of 5,5',6,6'-tetrahydroxy-3,3'-biindolyl, ferulic acid conjugates, phenolic amides, and several flavonoids was also reported (KUJALA et al., 2002). Glucosylated ferulic acid was found to be the clearly dominating phenolic compound in Swiss chard stalks (WINTER and HERRMANN, 1986) and further

Tab. 1: Total phenolics, ascorbic acid, and betalain contents as well as antioxidant capacities of extracts from differently coloured Swiss chard stems and beetroot hypocotyls

	Swiss chard stems				beetroots		
	white	yellow	orange	red-purple	white	yellow	red
total phenolics (as GAE[a]) [mg/100 g]	36.3 ± 0.1	50.0 ± 0.1	42.9 ± 0.3	31.2 ± 0.3	56.1 ± 0.1	41.5 ± 0.9	140.7 ± 0.1
total phenolics (as AAE[b]) [mg/100 g]	56.7 ± 0.2	78.3 ± 0.2	67.1 ± 0.4	48.6 ± 0.5	87.9 ± 0.2	65.0 ± 1.3	220.7 ± 0.1
ascorbic acid [mg/100 g]	–[c]	–	–	–	–	–	–
betaxanthins [mg/100 g]	–	5.9 ± 0.0	4.1 ± 0.0	2.1 ± 0.0	–	16.5 ± 0.1	53.3 ± 0.3
betacyanins [mg/100 g]	–	–	2.4 ± 0.0	3.6 ± 0.0	–	–	96.8 ± 0.5
total betalains [mg/100 g]	–	5.9	6.5	5.7	–	16.5	150.1
TEAC (as TE[d]) [µmol/100 g]	287.5 ± 7.8	429.0 ± 1.7	339.5 ± 2.5	252.4 ± 3.5	428.2 ± 0.6	362.1± 6.4	1110.3 ± 22.3
TEAC (as AAE) [mg/100 g]	44.0 ± 1.1	64.8 ± 0.2	51.6 ± 0.4	38.8 ± 0.5	64.7 ± 0.1	55.0 ± 0.9	167.6 ± 3.3
TEAC (as DAE[e]) [mg/100 g]	16.1 ± 0.4	23.9 ± 0.1	19.0 ± 0.1	14.2 ± 0.2	23.9 ± 0.0	20.2 ± 0.4	61.8 ± 1.2
FRAP (as TE) [µmol/100 g]	164.5 ± 3.7	273.6 ± 2.8	211.3 ± 2.5	263.7 ± 3.2	201.0 ± 4.1	221.0 ± 4.8	985.1 ± 5.5
FRAP (as AAE) [mg/100 g]	29.8 ± 0.7	49.2 ± 0.5	38.1 ± 0.4	47.9 ± 0.6	36.3 ± 0.7	39.8 ± 0.9	177.8 ± 1.0
FRAP (as DAE) [mg/100 g]	24.7 ± 0.5	40.8 ± 0.4	31.6 ± 0.4	39.7 ± 0.5	30.1 ± 0.6	33.0 ± 0.7	147.4 ± 0.8

[a] GAE = gallic acid equivalents
[b] AAE = ascorbic acid equivalents
[c] not detected
[d] TE = Trolox equivalents
[e] DAE = dopamine equivalents

derivatives of vitexin, kaempferol and isorhamnetin were reported to be present in Swiss chard leaves (GIL et al., 1998). It is worth being mentioned that the Folin-Ciocalteu reagent is not specific to phenolics, but will also react with a broad range of reducing compounds such as nitrogenous substances (IKAWA et al., 2003) including Dopa (3,4-dihydroxyphenylalanine), and ascorbic acid (SINGLETON et al., 1999). Moreover, betanin the major betacyanin in red beetroot exhibits a non-glycosylated hydroxyl group at C6 (Fig. 2) and is structurally similar to Dopa. Hence, it is conceivable that betanin will be assessed by the Folin-Ciocalteu method as supported by higher values with increasing pigment contents (Tab. 1). Furthermore, remarkable concentrations of free dopamine (KUGLER et al., 2006) and its corresponding betaxanthin (= miraxanthin V, Fig. 1, KUGLER et al., 2004), both exhibiting aromatic *ortho*-dihydroxyl substitution previously found in coloured Swiss chard stems, may likewise considered active. Although multiple food components may contribute to an overestimation of the true total phenolics content, the Folin-Ciocalteu assay is reliable, widely applied and has recently been suggested appropriate for the assessment of the total reducing strength *in vitro* (HUANG et al., 2005). Notwithstanding, the compositional pattern in food samples should be generally considered and the antioxidant data carefully interpreted accordingly.

In the Chenopodiaceae plant materials investigated in this study, no ascorbic acid could be detected, although up to 11 mg ascorbic acid/100 g red beetroot fresh weight and up to about 10 mg /100 g Swiss chard stalk fresh weight were reported elsewhere (HERRMANN, 1995; POKLUDA and KUBEN, 2002). GIL et al. (1998) pointed out that whole Swiss chard leaves exhibited around 45 mg vitamin C/100 g fresh weight, however, exclusively as dehydroascorbic acid. It is assumed that the same applied to the samples of the present study due to inevitable ascorbic acid oxidation during extraction. Therefore, ascorbic acid does not represent the major antioxidant in Swiss chard or red beetroot, but rather the colourless phenolics (KUJALA et al., 2000; 2002; WINTER and HERRMANN, 1986) and the betalains. Other electron-donors such as the catecholamine dopamine previously identified as a strong antioxidant (KANAZAWA and SAKAKIBARA, 2000) may also affect total antioxidant capacity. Since dopamine was found only recently in Swiss chard stems and beetroots ranging from 8.8 mg/100 g fresh weight in white Swiss chard stems to 36.6 mg/100 g fresh weight in red beetroot (KUGLER et al., 2006), antioxidant capacities determined by applying the TEAC and FRAP assays were also expressed as dopamine equivalents (Tab. 1). Due to high dopamine contents (KUGLER et al., 2006) and considerable antioxidant responses, dopamine was suspected to markedly contribute to the total antioxidant capacities of the Chenopodiaceae extracts investigated, although minor oxidative events converting dopamine into inactive dopaminequinone may still have occurred during extraction and sample work-up.
Based on a calibration with purified betanin, the relative contribution of betanin to the antioxidant activity in the FRAP assay was 3.9, 4.7,

and 33.7 % in orange Swiss chard petioles, red-violet Swiss chard petioles and red beetroot, respectively. The corresponding values in the TEAC assay amounted to 2.8, 5.7, and 34.1% and were thus quite similar to the FRAP values. The relatively small amount of betalains in Swiss chard may explain the comparatively low antioxidant capacities assessed.

Antioxidant capacities as determined by the TEAC assay (as TE) displayed excellent linear correlation with the respective total phenolics concentrations (as GAE) [$R^2 = 0.9962$]. Accordingly, maximal TEAC values were determined for red beetroot (1110.3 µmol TE/100 g fresh weight), yellow Swiss chard stems (429.0 µmol TE/100 g fresh weight) and white beetroot (428.2 µmol TE/100 g fresh weight). In a previous study, PELLEGRINI and co-workers (2003) reported a lower TEAC value of 521 µmol TE/100 g fresh weight for beet reaching about half of the total antioxidant capacity for red beetroot found in the present investigation. Additionally, PELLEGRINI et al. (2003) determined a total antioxidant activity of 353 µmol TE/100 g fresh weight for Swiss chard stems, thus being in the same range from 252.4 to 429.0 µmol TE/100 g fresh weight for red-purple and yellow coloured stems of the present study, respectively. Interestingly, the same authors (PELLEGRINI et al., 2003) found much lower TEAC values for carrots (44 µmol TE/100 g fresh weight) and eggplants (110 µmol TE/100 g fresh weight) containing carotenoids and anthocyanins as the predominant radical scavenging pigments, respectively.

Numerous publications stated excellent linear correlations between total phenolics contents and antioxidant capacities determined by electron transfer-based antioxidant capacity assays such as the TEAC and FRAP methods (HUANG et al., 2005). In the present work, an excellent linear correlation could be established for TEAC values (as TE) and total phenolics (as GAE) [$R^2 = 0.9962$], with the latter exhibiting a weaker correlation with FRAP values (see Tab. 1) [$R^2 = 0.9365$]. Although PROTEGGENTE et al. (2002) observed good correlations between total phenolics and both TEAC and FRAP values, they also noted that especially TEAC values were highly correlated with total phenolic contents, thus supporting the higher correlation coefficient in this study. Despite the mechanistic similarity of the TEAC and FRAP assays both being based on electron transfer reactions, the pH conditions in the two test systems are different, which might explain the differing rankings of the vegetables investigated (Tab. 1): Using the TEAC assay, the antioxidant activities of the investigated extracts were in the following order: red beetroot (rank 1) > yellow Swiss chard stems (rank 2) > white beetroot (rank 3) > yellow beetroot (rank 4) > orange Swiss chard stems (rank 5) > white Swiss chard stems (rank 6) > red-purple Swiss chard stems (rank 7). In contrast, when applying the FRAP assay, the antioxidant capacities ranked in the following order: red beetroot (rank 1) > yellow Swiss chard stems (rank 2) > red-purple Swiss chard stems (rank 3) > yellow beetroot (rank 4) > orange Swiss chard stems (rank 5) > white beetroot (rank 6) > white Swiss chard stems (rank 7). The fact that application of different assays may result in dissimilar orders of antioxidant capacities were already highlighted in a previous study (PELLEGRINI et al., 2003) with varying rankings for a broad range of vegetable extracts applying the TEAC and FRAP assays, respectively. Interestingly, in the present study, lowest FRAP values were found for betalain-free white Swiss chard stems and white beetroot hypocotyls, whereas all betalain-bearing samples were shown to possess higher FRAP antioxidant capacities with red beetroot displaying the maximum value of 985.1 µmol TE/100 g fresh weight. Noteworthy, previous investigations did not consider the betalains in betalainic plant material, but only the colourless phenolics which were exclusively held responsible for the *in vitro* bioactivities monitored (HALVORSEN et al., 2002; PELLEGRINI et al., 2003; PYO et al., 2004). In contrast, the present findings clearly support the data by WETTASINGHE et al. (2002), who reported a positive correlation between antioxidant activities and pigment contents of beetroots. According to ESCRIBANO et al. (1998), the betacyanins are more potent radical scavengers than the betaxanthins. This conception needs to be differentiated depending on the respective betaxanthin structures, because yellow Swiss chard petioles with miraxanthin V (Fig. 1) as major compound were found to be more potent than the red-purple ones with betanin (Fig. 2) as the predominant pigment at the same concentration level (Tab. 1). In fact, it has recently been demonstrated that betacyanins actually act as antioxidants *in planta* their synthesis being induced in beetroot leaves upon wounding and bacterial infiltration. It was suggested that following an oxidative burst the reactive oxygen species (ROS) generated were signals for the biosynthesis of betacyanins, the latter acting as ROS scavengers, thus limiting plant tissue damage (SEPÚLVEDA-JIMÉNEZ et al., 2004).
Although it is highly conceivable that betacyanins may act as antioxidants *in vivo*, it has to be considered that their efficacy in the human organism will depend on their bioavailability, which has been recently shown to be quite low (NETZEL et al., 2005). On the other hand, poor absorption has also been reported for the anthocyanins, despite their undisputed physiological activity (CLIFFORD, 2000; STINTZING and CARLE, 2004; GHOSH, 2005).

Cactaceae

In addition to coloured Swiss chard petioles and different beetroot varieties, cactus pear and pitaya fruit juices were included in this study (see Tab. 2). Maximum total phenolics concentration was determined for yellow-orange 'Gialla' juice (63.5 mg GAE/100 mL juice) only slightly differing from those obtained from the red cactus pear cultivar 'Rossa' (61.5 mg GAE/100 mL juice) and from the purple pitaya fruit species *H. polyrhizus* (61.0 mg GAE/100 mL juice). In striking contrast, the unpigmented juices from fruits of *H. undatus* and *S. megalanthus* contained only 18.2 and 22.7 mg GAE/100 mL juice, respectively. According to KUTI (2004), mainly quercetin and isorhamnetin conjugates and low amounts of kaempferol conjugates contribute to total phenolics of cactus pears while the pattern of phenolic compounds in pitayas has not been scrutinised yet.

For fruits of tropical origin cultivated on Mauritius island, LUXIMON-RAMMA and co-workers (2003) reported total phenolics to range from 11.8 to 563.8 mg GAE/100 g fresh weight for banana and red Chinese guava, respectively. Total phenolics for juices from cactus pears and pitaya fruits in the present study were in the same range (Tab. 2).
Considering the high ascorbic acid concentrations of juices from the cactus pear cultivars 'Gialla' and 'Rossa' with 31.5 and 23.9 mg/100 mL juice, respectively, and comparing them with total phenolics contents calculated as AAE, it was assumed that ascorbic acid markedly contributed to the total phenolics values measured. On the contrary, the juice from *H. polyrhizus* fruits was devoid of ascorbic acid, and only minute amounts were found in juices obtained from fruits of *H. undatus* and *S. megalanthus*, respectively (Tab. 2). Thus, the contribution of ascorbic acid to the total phenolics value in pitaya juices was negligible. Nevertheless, the high betacyanin concentration of 46.0 mg/100 mL juice contributed distinctively as already suspected by WU and co-workers (2006). In the TEAC and FRAP assays, the particular contribution of ascorbic acid was 42.8 and 68.0% for 'Gialla', 34.6 and 52.8% for 'Rossa', 7.0 and 15.0% for *H. undatus*, and 2.6 and 6.0 % for *S. megalanthus*. Based on a calibration with purified Betanin, the particular activity of betacyanins as betanin-equivalents amounted to 0.5, 5.0, and 45.2% in 'Gialla', 'Rossa' and *H. polyrhizus* in the TEAC assay, respectively. The corresponding values in the FRAP test were slightly different with 0.8, 8.1, and 36.0%, respectively.
Again, the values obtained for juices from cactus fruits in the present study were consistent with the previously determined values for tropical fruits ranging from 0.8 to 142.6 mg ascorbic acid/100 g fresh weight for banana and white guava, respectively (LUXIMON-RAMMA et al., 2003).

Tab. 2: Total phenolics, ascorbic acid, and betalain contents as well as antioxidant capacities of cactus fruit juices

	cactus pear cultivars		pitaya species		
	'Gialla'	'Rossa'	*H. polyrhizus*	*H. undatus*	*S. megalanthus*
total phenolics (as GAE[a]) [mg/100 mL]	63.5 ± 1.1	61.5 ± 0.9	61.0 ± 0.8	18.2 ± 0.3	22.7 ± 0.2
total phenolics (as AAE[b]) [mg/100 mL]	99.5 ± 1.7	96.4 ± 1.4	95.7 ± 1.2	28.2 ± 0.5	35.3 ± 0.3
ascorbic acid [mg/100 mL]	31.5 ± 0.0	23.9 ± 0.4	–[c]	2.0 ± 0.1	0.8 ± 0.0
betaxanthins [mg/100 mL]	4.8 ± 0.0	3.3 ± 0.0	–	–	–
betacyanins [mg/100 mL]	0.6 ± 0.0	5.9 ± 0.0	46.0 ± 0.3	–	–
total betalains [mg/100 mL]	5.4	9.2	46.0	–	–
TEAC (as TE[d]) [µmol/100 mL]	488.5 ± 10.7	457.6 ± 4.6	411.6 ± 22.2	181.5 ± 1.6	201.5 ± 3.8
TEAC (as AAE) [mg/100 mL]	73.6 ± 1.6	69.0 ± 0.7	63.7 ± 3.3	28.4 ± 0.2	30.5 ± 0.6
FRAP (as TE) [µmol/100 mL]	254.7 ± 0.7	249.4 ± 4.0	438.0 ± 1.9	72.3 ± 1.9	73.9 ± 1.4
FRAP (as AAE) [mg/100 mL]	46.3 ± 0.1	45.3 ± 0.7	79.0 ± 0.3	13.3 ± 0.3	13.4 ± 0.2

[a] GAE = gallic acid equivalents
[b] AAE = ascorbic acid equivalents
[c] not detected
[d] TE = Trolox equivalents

As observed for the Chenopodiaceae, again a good linear correlation of TEAC values (as TE) with the corresponding total phenolics contents (as GAE) [$R^2 = 0.9764$] was registered for the cactus fruit juices (Tab. 2). Accordingly, the highest TEAC value (488.5 µmol TE/100 mL juice) was determined for 'Gialla' juice, followed by 'Rossa' (457.6 µmol TE/100 mL juice) and *H. polyrhizus* (411.6 µmol TE/100 mL juice), respectively. Less than half of the former antioxidant capacities was detected in the colourless juices from *H. undatus* and *S. megalanthus* fruits only amounting to 181.5 and 201.5 µmol TE/100 mL juice, respectively. Being in a similar range, STINTZING and co-workers (2005) reported TEAC values for several cactus pear clones ranging from 310 to 499 µmol TE/100 mL juice. By comparison with TEAC values obtained for extracts from Mauritian fruits (LUXIMON-RAMMA et al., 2003), antioxidant capacities of the investigated cactus fruit juices may be classified as follows: A portion of 100 mL of juice from fruits of the cactus pear cultivars 'Gialla' and 'Rossa' or the pitaya species *H. polyrhizus* ranks at a similar level as 100 g of edible parts from mango and litchi fruits (500 µmol TE/100 g fresh weight), respectively. Displaying lower total antioxidant capacities, juices from the pitaya fruits *H. undatus* and *S. megalanthus* rank at a level comparable to pineapple, avocado, and jamalac fruits (200 µmol TE/100 g fresh weight), respectively.

Comparing the TEAC values for cactus fruit juices assessed in this study and the mean TEAC value for commercial blood orange juice (282 µmol TE/100 mL) being rich in potent antioxidative anthocyanins (FIORE et al., 2005), it appears that betalainic juices from fruits of the cactus pear cultivars 'Gialla' and 'Rossa' as well as from the pitaya species *H. polyrhizus* exert even stronger antioxidant activities whilst both the colourless juice from fruits of the pitaya species *H. undatus* and *S. megalanthus* display weaker antioxidant capacities than blood orange juice (Tab. 2). Only recently, red-fleshed 'Weirouge' apples being rich in anthocyanins were assessed and exhibited total antioxidant capacities of 585 and 240 µmol TE/100 g whole apple edible portion in the TEAC and FRAP assays, respectively (SADILOVA et al., 2006). Comparing these results with the values obtained for cactus fruit juices in the present investigation, it becomes again evident that antioxidant capacity rankings strongly depend on the respective sensitivity to the particular compound class: Whilst in the TEAC assay, 100 g of unpeeled 'Weirouge' apple ranks best, juice from the pitaya fruit species *H. polyrhizus* possesses the highest total antioxidant capacity based on the values obtained by the FRAP assay.

As has been observed for the extracts from Swiss chard petioles and beetroot hypocotyls (Tab. 1), FRAP values (as TE) of cactus fruit juices only poorly correlated with the respective total phenolics concentrations (as GAE) [$R^2 = 0.7245$, linear fit]. The juice from fruits of *H. polyrhizus* containing phyllocactin and hylocerenin as major betacyanins (Fig. 2) displaying an extraordinary betacyanin content (46.0 mg betacyanins/100 mL juice), exhibited the highest FRAP value (438.0 µmol TE/100 mL juice), followed by 'Gialla' (254.7 µmol TE/100 mL juice) and 'Rossa' juice (249.4 µmol TE/100 mL juice), with the latter both only slightly differing in their betalain contents (Tab. 2). Accordingly, lowest FRAP values were determined for the betalain-free juices from fruits of *S. megalanthus* (73.9 µmol TE/100 mL juice) and *H. undatus* (72.3 µmol TE/100 mL juice).

Despite their low phenolic contents, previous studies attributed the antioxidant potential of cactus pears mainly to the presence of phenolic compounds (GALATI et al., 2003; 2005; KUTI, 2004) and only recently, the reducing capacities of betanin and indicaxanthin from cactus pear fruits were unambiguously proven (BUTERA et al., 2002). Moreover, TESORIERE and co-workers (2004) showed that the cactus pear betalains indicaxanthin and betanin may be involved in the protection of low density lipoproteins (LDL) against *ex vivo*-induced oxidative modifications. As mentioned before, efficacy of cactus fruit betalains in the human organism will depend on the extent of absorption.

In addition to total phenolics, ascorbic acid, and betalains, further compounds present in cactus juices should be taken into account when evaluating overall bioactivity. In this regard, high proline levels were recently reported (KUGLER et al., 2006) in juices from the cactus pears 'Gialla' (1476.9 mg proline/kg juice) and 'Rossa' (1837.2 mg proline/kg juice), as well as pitaya fruits from *S. megalanthus* (1803.7 mg proline/kg juice), *H. polyrhizus* (273.3 mg proline/kg juice), and *H. undatus* (254.5 mg proline/kg juice). Until now, proline was mainly assumed to be accumulated in plants as osmolyte to withstand drought and high salinity (STINTZING and CARLE, 2004). Although proline itself did not exert antioxidant effects neither in the TEAC nor in the FRAP assays (data not shown), it was only recently assumed to act as ROS scavenger in the fungal pathogen *Colletotrichum trifolii* (CHEN and DICKMAN, 2005). Possibly, proline acts in a synergistic way with other antioxidant compounds of cactus fruit juices. Further studies are re-

quired to elucidate the role of proline as potential antioxidant. Besides proline, biothiols, vitamin E, as well as carotenoids may additionally contribute to the total antioxidant capacities of cactus juices (TESORIERE et al., 2005). Furthermore, taurine (2-aminoethane-sulfonic acid) earlier found in cactus pear juices in a range from 32-57 mg/100 mL (STINTZING et al., 1999) or 8-12 mg/kg (TESORIERE et al., 2005) should be considered because MILITANTE and LOMBARDINI (2004) assumed that taurine may exert antioxidant effects.

Conclusions

The present investigation demonstrated that despite the similar underlying mechanisms, total antioxidant capacities of betalainic samples depend on the respective assay applied. The extract from red beetroot containing large amounts of the betacyanin betanin exhibited the highest antioxidant capacity among the Chenopodiaceae representatives in both test systems, whilst betacyanin-rich juice from fruits of the pitaya species *H. polyrhizus* showed highest antioxidant capacity within the Cactaceae samples only in the FRAP assay. Additionally, it has to be taken into account that the data obtained from antioxidant capacity assays are not only influenced by the particular methods, but also the compositional pattern of the respective crop being affected by the geographical origin as well as harvest time of the plant materials investigated (OU et al., 2002). Altogether, the present study confirmed that foodstuffs containing significant amounts of betalains do not only represent colouring potential but are also excellent sources of dietary antioxidative phytochemicals which may exert beneficial effects on consumer's health upon consumption that remain to be carefully scrutinised.

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