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RELATIONSHIP BETWEEN ANTIOXIDANT CAPACITY OF RED BEET JUICE AND CONTENTS OF ITS BETALAIN PIGMENTS

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Juices extracted from red beet roots were analysed in the study. They were produced from 7 different batches of cv. Czerwona Kula from the harvest of 2002 and 11 different cultivars cultivated under identical conditions in 2005. Contents of red and yellow pigments as well as antioxidant capacity were determined in juices. A very wide variation was observed in antioxidant capacity (10.2–21.7 μmol Trolox/mL) and contents of red pigments (0.57–1.63 mg/mL) and yellow pigments (0.31–0.95 mg/mL). In comparison to other vegetables, the antioxidant capacity of beets was very high. A highly significant correlation was demonstrated between antioxidant capacity and contents of red pigments, whereas a remarkably less tangible relationship was found between antioxidant capacity and contents of yellow pigments. This indicates that primarily red betanin is responsible for the antioxidant capacity of red beets.

INTRODUCTION

Red beets contain betalain pigments, belonging to the group of cation antioxidants [Kanner *et al.*, 2001]. Betalains are coloured, water-soluble nitrogen compounds, found in the cell sap. Betalain pigments are nitrogen pigments present in most families of the order *Caryophyllales* and higher fungi. The best known edible plants containing betalains are red beets and cactus fruit, such as prickly pears and *Hyloceerus polyrhisus* [Frank *et al*., 2005].

Betalain pigments are classified into two groups: red betacyanins and yellow betaxanthines. All betalains contain betalamic acid, a substituted dihydropyridine with a system of conjugated double bonds. Betalamic acid may condense either with cyclo-3-(3,4-dihydroxyphenyl)-l-alanine (cyclo-DOPA), which may be glycosylated, to produce betacyanins, or with various amino acids or amine derivatives to produce betaxanthins. Betanidin is immonium conjugate of betalamic acid and cyclo-DOPA. The red betanine, betanidin 5-O-beta-glucoside, and yellow vulgaxanthine I, the adduct of betalamic acid and glutamine, are the main betalain pigments in red beet [Nilsson, 1970].

Betalains show antioxidant and radical scavenging activities [Pedreno & Escribano, 2000; Gliszczyńska-Świgło *et al.*, 2006; Allegra *et al.*, 2007]. They are bioavailable for the human organism as they permeate from the alimentary tract to the blood stream [Netzel *et al.*, 2005]. The antioxidant properties of betacyanins are attributable to their free phenolic groups. The cyclic amine group of the betalamic acid moiety is considered to act as a hydrogen donor both in betacyanins and betaxanthins [Kanner *et al.*, 2001].

The aim of the study was to assess the relationship between antioxidant capacity of red beet juice and contents of its red and yellow pigments. The experiments were conducted on material varying in terms of its origin and cultivar.

MATERIALS AND METHODS

Plant material

Juices were produced from roots of red beet cv. Czerwona Kula from the harvest of 2002 and from 11 cultivars from the harvest of 2005. Beets of cv. Czerwona Kula were purchased at different shops in the city of Poznań in January 2003. Beets were declared to be cv. Czerwona Kula by the seller and morphological characteristics of roots were consistent with data reported for this cultivar [Dobrakowska-Kopecka *et al.*, 1989]. Roots of 11 red beet cultivars, *i.e.* Ceryl, Chrobry, Czerwona Kula, Nochowski, Noe 21, Noe 694, Noe 904, Noe Pol, Okrągły Ciemnoczerwony, Opolski, and Wodan, were supplied by the „Spójnia" Plant Breeding, Seed Production and Horticultural Station in Nochowo, Poland, from the crop harvested in 2005. Identical cultivation and growth conditions during vegetation were maintained for the raw material.

Juice production

Juice was pressed from 2 kg of beetroots using a Zelmer juice extractor, while solids were separated from juices by centrifugation at 10,000 ×*g* for 15 min.

Chemicals

2,2'azino-bis-(3-ethylbenzothiazoline-6-sulfonic acid (ABTS), 6-hydroxy-2,5,7,8,-tetramethyl-chroman-2-carboxylic acid (Trolox)

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and di-potassium peroxidisulfate ($K_2O_8S_2$) were purchased from Sigma-Aldrich L.L.C. (Poznań, Poland). All other chemicals were purchased from P.O.CH S.A (Gliwice, Poland) and were all of analytical grade.

Determination of total betalain pigments contents

Betalain pigments were assayed by differential spectrophotometry according to Nilsson [1970]. Juice was diluted with a phosphate buffer (pH 6.5) and absorbance was measured at 476 nm for yellow pigments and at 538 nm for red pigments using a Helios Alfa spectrophotometer (Thermo Electron Corporation, USA).

Trolox Equivalent Antioxidant Capacity (TEAC) assay

TEAC of juices was determined according to Re *et al*. [1999] using a Helios Alfa spectrophotometer with a temperature controlled cell holder (Thermo Electron Corporation, USA) and expressed as μmol Trolox equivalent/mL. Juice was diluted with a phosphate buffer and measurements were taken 6 min after it was mixed with ABTS cation radical solution.

Statistical analysis

The data were evaluated statistically using STATISTICA ver. 7 software package. The results were analysed statistically to establish mean values, standard deviations and correlation coefficients. The analysis of regression between contents of pigments and TEAC value of beet juices was performed using the least squares method.

RESULTS

High variation in contents of pigments and antioxidant capacity was observed in juices of cv. Czerwona Kula beets from harvest of 2002 (Table 1). Contents of red pigments ranged from 0.57 to 1.37, and those of yellow pigments – from 0.40 to 0.61 mg/mL, respectively. Moreover, the antioxidant capacity fell within a wide range from 10.6 to 21.7 μmol Trolox/mL.

Contents of pigments and antioxidant capacity of juices extracted from different beet cultivars from Nochowo analysed in 2005 varied considerably, depending on the cultivar (Table 2). In these juices, contents of red pigments ranged from 0.62 to 1.63, while contents of yellow pigments varied from 0.31 to 0.95 mg/mL. TEAC values were also highly varied, ranging from 10.2 to 20.6 μmol Trolox/mL. The highest content of red pigments was found in juices produced from cv. Chrobry and Nochowski. Juices extracted from these cultivars exhibited also high TEAC values.

A positive correlation was tangibly manifested between TEAC values and contents of red pigments, observed both for individual series of assays and when such a correlation was assessed for all samples (Table 3). Considerably lower correlation coefficients were observed between antioxidant capacity and contents of yellow pigments. In the case of juices produced from cv. Czerwona Kula harvested in 2002, this correlation was not statistically significant.

For juices of cv. Czerwona Kula beets, the correlation between contents of red and yellow pigments was non-significant. In contrast, a highly significant positive correlation was recorded in the case of cultivars from Nochowo (Table 3). A highly significant correlation at $p<0.01$ was also observed between these variables for all analysed samples.

It needs to be stressed that the regression line for the relationship between TEAC values and contents of pigments did not intersect with the coordinate axis at point 0 (Table 3). The positive value of the shift coefficient for the regression line indicates that apart from pigments, juices contain also other antioxidant compounds.

TABLE 1. Pigments content and antioxidant capacity of beetroot juice – Czerwona Kula cultivar, harvest year 2002. Means from 3 replicates ±S.D.

Sample	Red pigments (mg/mL)	Yellow pigments (mg/mL)	Antioxidant capacity (μmol Trolox/mL)
1	0.57±0.004	0.46±0.006	10.6±0.52
2	0.59±0.007	0.51±0.010	12.6±0.29
3	0.68±0.004	0.40±0.011	12.7±0.10
4	0.80±0.005	0.42±0.008	13.3±0.09
5	0.94±0.005	0.60±0.010	14.9±0.20
6	1.36±0.006	0.61±0.028	21.3±0.08
7	1.37±0.006	0.44±0.028	21.7±0.08
Mean	0.90	0.49	15.3
S.D. for mean	0.34	0.09	4.42

DISCUSSION

Results indicate a very high variation in contents of pigments and TEAC value between cultivars (Table 2), as well as different batches of beets of the same cultivar coming from different sources.

Contents of betacyanins in beet roots fall within a very broad range of values. According to Nilsson [1973], betacyanin contents range from 0.30 to 2.2 mg/g fresh weight, and may even reach 3 mg/g [Sobkowska *et al.,* 1975]. The content

TABLE 2. Pigments content and antioxidant capacity of beetroot juice – 11 cultivars from Nochowo 2005. Means from 3 replicates ±S.D.

Cultivar	Red pigments (mg/mL)	Yellow pigments (mg/mL)	Anioxidant capacity (μmol Trolox/mL)
Ceryl	1.17±0.005	0.95±0.001	18.8±0.19
Chrobry	1.63±0.006	0.70±0.008	20.6±0.21
Czerwona Kula	0.62±0.002	0.31±0.001	10.2±0.26
Nochowski	1.40±0.005	0.91±0.008	18.0±0.18
Noe 21	1.19±0.003	0.54±0.004	16.9±0.16
Noe 694	0.71±0.005	0.33±0.003	13.3±0.31
Noe 904	1.27± 0.004	0.64±0.003	19.2±0.30
Noe Pol	0.67±0.004	0.46±0.003	13.0±0.27
Okrągły Ciemnoczerwony	1.13±0.003	0.53±0.004	17.4±0.31
Opolski	0.89±0.003	0.60±0.003	15.9±0.31
Wodan	0.74±0.003	0.52±0.003	13.6±0.21

TABLE 3. Summary of a linear correlation and regression for pigments contents and antioxidant capacity (TEAC – Trolox equivalent antioxidant capacity).

Object	N	Independent variable x	Dependent variable y	Regression equation	R	p value for regression
Czerwona Kula cultivar, 2002	7	red pigments	yellow pigments	y=0.098x+0.1	0.389	0.388
		red pigments	TEAC	y=0.129x+3.70	0.985	0.000
		yellow pigments	TEAC	y=0.192x+5.94	0.370	0.415
Cultivars from Nochowo, 2005	11	red pigments	yellow pigments	y=0.448x+12.69	0.726	0.011
		red pigments	TEAC	y=0.090x+6.75	0.939	0.000
		yellow pigments	TEAC	y=0.121x+8.98	0.772	0.005
All samples	18	red pigments	yellow pigments	y=0.330x+22.51	0.637	0.004
		red pigments	TEAC	y=0.103x+5.66	0.946	0.000
		yellow pigments	TEAC	y=0.123x+9.00	0.587	0.010

of betaxanthines also varies considerably and ranges from 0.2 to 1.4 mg/g [Nilsson, 1973]. The level of betalain pigments depends on many factors, such as the size of roots, cultivar, climatic and agricultural conditions [Mazza & Chubey, 1985; Goldman, 1995].

Antioxidant capacity of juices may only be compared with data given by Mikołajczyk & Czapski [2006], according to whom the antioxidant capacity in beets after harvest ranged from 12.0 to 27.7 μmol Trolox/g. In this study, analyses were performed for another batch of supplied beets.

On the basis of regression analysis it may be stated that there is a very close relationship between the content of red pigments and antioxidant capacity of red beet. Values of correlation coefficients for these dependencies were very high, which indicates that the TEAC value of red beet juice depends primarily on the contents of red pigments.

A regression coefficient computed for the dependence of the antioxidant capacity on contents of yellow pigments was much higher than in the case of such a dependence for red pigments (Table 3). This results – among other things – from the fact that contents of yellow pigments were on average 1.8 times lower than these of the red pigments. It also needs to be pointed out that contents of yellow pigments were correlated with contents of red pigments.

There is no comparison available in literature sources concerning the antioxidant activity of betanin and vulgaxanthines, pigments typical of red beets. According to Escribano *et al*. [1998], betanin has a higher antioxidant activity than vulgaxanthine, but data reported by those authors were incomplete.

According to Borowska [2003], the antioxidant capacity of beet roots is 8.1 μmol Trolox/g. As it was stated in that study, the antioxidant capacity of beet juice is strongly correlated with contents of red pigments. The high antioxidant capacity of cultivars analysed in this study was due to their high pigments contents. Moreover, it may also be stated that red beets in this respect rank very high among vegetables, their position being comparable to that of spinach, Brussels sprouts or kale [Chu *et al.,* 2002].

The high rank of many species of fruit and vegetables in terms of their antioxidant capacity results from their high contents of phenolic compounds. Contents of flavonoids and phenolic acids in beets are very low. The content of ferulic acid derivatives in the parenchyma of red beets ranges from 0.05 to 0.20 mg/g dry matter, *i.e.* approx. 0.01–0.04 mg/g fresh weight [Kujala *et al.,* 2002]. In the skin. the content of ferulic acid derivatives is approx. 1 000 times lower than that of betanin [Kujala *et al.,* 2000]. This shows that primarily betalains are responsible for the antioxidant capacity of red beets.

CONCLUSIONS

A high variation is observed in juices extracted from red beet in terms of their antioxidant capacity and contents of red and yellow pigments. There was a highly significant correlation between antioxidant capacity and contents of red pigments, whereas a remarkably less tangible relationship was found between antioxidant capacity and contents of yellow pigments. Antioxidant capacity of red beets may easily be assessed indirectly on the basis of a simple spectrophotometric measurement of red pigments content.

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