Correlation between pigment contents and FRAP values in beet root (Beta vulgaris ssp. esculenta var. rubra)

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Summary: It is well known that beetroot quality is determined mainly by the red pigment content (betacyanins) and its uniformity of the root. The effect of the most important red pigment components (betanin). the total polyphenol content and antioxidants were studied in 20 beet root varieties. Antioxidants were expressed in FRAP (ferric reducing ability of plasma) values in μM/l.

Our results indicated a close correlation (r = 0.7799 and r = 0.7435. respectively) between betanin and total polyphenol contents of the root as well as between FRAP values.

Our measurements showed more than threefold differences in total antioxidant activity among varieties. the lowest value being 196.4 μM/l and highest 702.57 μM/l. The corresponding betanin (16.3 and 57.8 mg/100 ml) and total polyphenol (37.5 and 85.5 mg/100 ml respectively) contents show similar differences. Based on our results it can be stated that varieties of higher betanin and poliphenol contents have higher antioxidant values as well.

Accordingly, the two compounds must have a role in the evolution of antioxidant effects.

Key words: beetroot varieties, betanin content, FRAP, polyphenol, antioxidant activity

Introduction

Free radicals derived from oxigen play an important part in the pathomechanism of different illnessis. Living organisms are supplied with an effective defence system against oxigen radicals. The first defense line is composed of antioxidant enzymes but different vitamins and low molecule compounds, such as phenols, thiols and flavonoids, are also effective against radicals. These compounds are found in high quanities in vegetables. Juice made of numerous fruit and vegetables contain antioxidants compounds in different amount *(Steinberg, 1991)*. These compounds are mostly of polyphenol type and are able to bind free radicals and protect from the oxidation of biological molecules, membranes and tissues induced by active oxigen and free radicals *(Sies, 1991)*.

The anti-tumour effect of beta carotene found in carrots in large quanities has largely discussed in several publications as for its effect on tumours induced by UV-B.

Coloured componds are likely to possess higher anti-tumour effects than white or colourless ones *(Hoff et al., 1981)*. *Pedreno et al., (1999)* stated that the FRAP ability of beet root red components (betacyanins) is higher than that of the yellow ones (betaxantins).

Rasic et al (1983) utilised the antioxidant effect of beet root in their trials with lactic acid fermented beet rootjuice to decrease the prolification of tumor cells considerably. *Ferenczi (1986)* used the pressed juice of raw beet roots succesfully to treat tumor illnesses in human therapy.

The usability of table beet root in this way is, naturally, affected by its inner colour intensity as well. Our former studies proved considerable differences in colours and mineral contents among varieties *(Takácsné Hájos, 1997, Takácsné Hájos et al., 1999)*.

Our trial aimed at estabilishing what compounds could be correlated with the favourable physiological effects of beet root. Their quantity and dependence on genotype were also studied.

Material and method

Trials of OMMI included 20 beet root varieties (*Table 1*) sown in sandy soil at Nagykőrös, with 40 cm row distance, on 2nd April 2002.

Table 1 Trial of beet root varieties, Nagykőrös, 2002.

Name of variety	Root type	Origin
1. Bonel	spherical	Nickerson Zwaaan
2. Libero	spherical	Rijk Zwaan
3. Cylindra	cylindrical	Seminis
4. Metauro	spherical	Rijk Zwaan
5. Little Ball	spherical	Syngenta
6. Regala	spherical	Bejo
7. Bikores	spherical	Bejo
8. Rocket	cylindrical	Bejo
9. Bordó	spherical	ZKI
10. Bolivar	spherical	Nunza
11. Detroit	spherical	GUMO Bt.
12. Tűzgolyó	spherical	ZKI
13. Bíborgömb	spherical	ZKI
14. Nero	spherical	Seminis
15. Bíborhenger	cylindrical	ZKI
16. Favorit	spherical	ZKI
17. Rubin	spherical	ZKI
18. Pablo	spherical	Bejo
19. Pronto	spherical	Bejo
20. Forono	cylindrical	Daehnfeldt

After harvesting (20 June 2002) pigment content, total polyphenol and dry matter contents, and within morphological evaluation the shape of root and thickness of bottom root were analysed in field and laboratory.

Inner colour intensity and uniformity were evaluated organoleptically (scores 1–5).

Samples represented 10–12 roots.

The red pigments (betacyanins)were evaluated from diluted samples with HPLC at λ=538 nm and the pigment quantity was calculated in relation to betanin – the component of the highest quantity (mg/100 g) – by means of $E_{1\%}$ = 1120 specific extinction coefficient (*Cai et al., 1998; Forni et al., 1992*).

A spectrophotometer was used to determine the absorbance of pigments: λ=538 nm for red pigments and λ=476 nm for yellow ones.

For total phenol content the colour reaction to Folin-Denis reagents were evaluated at λ=760nm, by means of a katechin standard (mg katechin/100 ml) (*AOAC, 1990*).

Total antioxidant content was expressed in the so-called FRAP values (ferric reducing ability of plasma) in µM/l. The method is based on the ability of antioxidants to reduce Fe(III) ions to Fe(II) ions in buffered sour medium (pH 3.6). The produced Fe (II) can be measured on photometers. Absorbance is proportional to the quantity of the produced Fe(II) ions and the antioxidants, respectively.

The effect of antioxidants was determined according to *Benzie-Strain (1996)*. The method is based on the effect of antioxidants to be able to reduce the Fe (III) ions to Fe (II) ions in a complex compound (Fe(III) – trypiridine triazine). The produced Fe(II) compound is blue-coloured and its intensity is measured at 593 nm. After measuring the absorbance of the blue-coloured solution the concentration can be determined using the calibration curve made by known Fe(II) solutions.

Table 2 Morphological and quality indices of beet root varieties, Szarvas, 2002.

Variety	Root shape	Bottom root thickness	Colour uniformity 1 – white ring 5 – uniform	Colour intensity 1 – carmine 5 – deep purple	Taste 1– bitter, earthy 5 – sweet
1. Bonel	spherical	thin	4	5	5
2. Libero	spherical	thin	4	5	3.5
3. Cylindra	cylindrical	thick	1	3	2
4. Metauro	spherical	thin	4.5	4	4
5. Little Ball	spherical	thin	3	5	2
6. Regala	spherical	very thin	4.5	5	3
7. Bikores	spherical	thin	5	5	2
8. Rocket	cylindrical	thin	3	4	4
9. Bordó	spherical	thick	4	4	4
10. Bolivar	spherical	mid-thick	4.5	4.5	4.5
11. Detroit	spherical	thick	3	4	4
12. Tűzgolyó	spherical	mid-thick	3.5	3.5	2
13. Bíborgömb	spherical	thick	2	4	4.5
14. Nero	spherical	thin	3	5	4.5
15. Bíborhenger	cylindrical	mid-thick	2	4	2.5
16. Favorit	spherical	thick	4	5	4
17. Rubin	spherical	thin	4.5	5	4.5
18. Pablo	spherical	very thin	5	5	4
19. Pronto	spherical	thin	5	5	4
20. Forono	cylindrical	thin	3	4.5	5

Results and evaluation

Beet root quality is considerably influenced by inner root colour and uniformity. They are evaluated first organoleptically. The processing industry also uses sensory methods beside laboratory tests at the reception of raw material.

Results are shown in *Table 2*. Considerable differences are found not only in colour intensity but also in uniformity among varieties.

The thickness of the bottom root determines the loss caused by cleaning. Thicker bottom roots are more common in cylindrical varieties (3, 15) but are also found in varieties of spherical shape (9, 11, 16). Beside natural conditions this character is genetically fixed. In the tested varieties numbers 7, 17, 18 and 19 were the best in this respect.

A peculiar taste of root is caused by geozmin produced by symbiotic microorganisms. This compound is responsible for the disagreeable earthy, musty taste of the root. Sugars are also very important compounds adding to the comsumption value especially when eaten raw. The processing industry also prefers high sugar content as less sugar is needed to make pickles.

Sensory trials indicated No. 1 and 20 to be the best in taste.

In the quantitative determination of beet root pigment quality the amount of betanin, present in the highest quantity of red components was measured as well as the absorbance values for the quantity of total red and yellow pigments at the given wave length *(Table 3)*.

The highest betanin content (above 50 mg/100 g) was measured in the varieties 1, 7, 10, 13, 18 and 19. In these varieties the absorbance values were also very high. The close correlation between the two measurement results (r=0.768) proves that absorbance values alone – without precise betanin quantity determination – can qualify the given raw material. Thus, in a routin test of receving the raw material, satisfying information can be obtained from photometric analysis without using expensive HPLC methods.

The evolution of absorbance values for yellow and red pigments showed similar tendencies in the different varieties as also proved by the close correlation between them (r=0.899), which can be explained by the common initiative compound (betalam acid) of the two pigment groups (betacyanins and betaxanthins). There is a direct relation between the biosynthesis path of the two pigments.

Correlation between antioxidant activity of different varieties, pigment and total phenol contents are shown in *Table 4*.

Table 3 Pigment composition and quantitative occurrence of red components in beet root varieties Szarvas, 2002.

Variety	Betanin content (mg/100 g)	Absorbance values		
		red	yellow	ratio
		(λ=538 nm)	(λ=476 nm)	
1. Bonel	51.3	1.081	0.708	1.527
2. Libero	41.5	0.973	0.746	1.304
3. Cylindra	16.3	0.425	0.295	1.441
4. Metauro	35.3	0.815	0.599	1.360
5. Little Ball	33.1	0.832	0.600	1.387
6. Regala	39.1	0.800	0.541	1.479
7. Bikores	57.9	0.990	0.703	1.408
8. Rocket	44.2	0.865	0.573	1.510
9. Bordó	45.2	1.000	0.570	1.754
10. Bolivar	51.2	0.979	0.684	1.431
11. Detroit	30.8	0.841	0.487	1.727
12. Tűzgolyó	39.9	0.784	0.520	1.507
13. Bíborgömb	68.7	0.825	0.591	1.396
14. Nero	36.4	0.768	0.495	1.552
15. Bíborhenger	17.8	0.382	0.263	1.452
16. Favorit	36.0	0.754	0.503	1.499
17. Rubin	39.9	0.692	0.503	1.376
18. Pablo	51.7	1.030	0.693	1.486
19. Pronto	57.8	0.996	0.879	1.133
20. Forono	41.4	0.797	0.580	1.374
Varietal mean:	**40.26**	**0.831**	**0.577**	**1.440**

– correlation between betanin content and red pigment absorbance r = 0.768
– correlation in absorbance between red and yellow pigents r = 0.899

Table 4 FRAP values and quantity indices of beet root varieties Szarvas, 2002.

Variety	Betanin content (mg/100 g)	Total poliphenol content (mg katechin/100 ml)	FRAP values (μM/l)	Dry matter content (%)	Mg content (mg/kg)
1. Bonel	51.3	72.0	506.97	7.83	1801
2. Libero	41.5	63.5	352.11	8.50	2499
3. Cylindra	16.3	37.5	196.40	12.50	1537
4. Metauro	35.3	65.0	352.39	11.69	2311
5. Little Ball	33.1	63.5	313.27	11.70	1492
6. Regala	39.1	53.5	365.54	10.10	1565
7. Bikores	57.9	82.0	408.09	11.28	2027
8. Rocket	44.2	64.0	329.84	9.31	3362
9. Bordó	45.2	71.5	278.96	13.77	1887
10. Bolivar	51.2	78.5	359.69	11.34	1904
11. Detroit	30.8	54.0	280.57	13.08	1278
12. Tűzgolyó	39.9	63.5	413.29	12.41	2567
13. Bíborgömb	68.7	113.0	318.43	11.72	2061
14. Nero	36.4	61.5	378.14	11.15	1849
15.Bíborhenger	17.8	1.5	171.13	9.34	2644
16. Favorit	36.0	58.0	343.57	12.8	1990
17. Rubin	39.9	65.0	363.29	10.23	2100
18. Pablo	51.7	74.0	571.43	10.88	1878
19. Pronto	57.8	85.5	702.57	9.69	1888
20. Forono	41.4	60.5	457.00	11.36	2231
Varietal mean	40.26	63.03	373.13	11.03	1043.55

Correlation value (r = 0.7577) between betanin and total polyphenol contents
Correlation value (r = 0.7799) between betanin and FRAP values
Correlation value (r = 0.7435) between total polyphenols and FRAP values

The betanin and total polyphenol content of different genotypes show similar tendencies as proved by the close correlation between the two parameters (r=0.7577).

It is well known that besides pigments phenols also take part in antioxidant effects. Proof is the close correlation between betanin content and FRAP values (r=0.7799) and the correlation between total polyphenols and FRAP values (r=0.7435).

High differences were found in the Mg-content of varieties.

The presence of this element, however, cannot be connected directly the antioxidant effect of table beet root. The Mg-supply depends on the combined effect of several environmental an genetical factors.

Our results confirm former statements that betacyanins *(Pedreno et al., 1999)* and polyphenols *(Sies, 1991)* play an important part in the development of free radical capacity of table beet root.

Unfortunately, no correlation was found between dry matter content and betacyanin quantity

(r = - 0.286). Roots of higher pigment and dry matter contents are to be favoured as tumour inhibiting effect of lactic acid fermented beetroot juice is correlated with higher dry matter content by *Rasic et al. (1983)*.

Discussion

A test of 20 beetroot varieties revealed that the intense and uniform red colour of the root is associated with higher betanin content.

There is a close correlation between red pigments (betanin) and total polyhenol contents (r=0.7577).

The correlation between the quantity of these compounds and the FRAP values (r=0.7799 and r=0.7435, respectively) indicate the decisive part of pigments in the nutritional effect of a given variety.

Among varieties more than threefold differences were found in antioxidant activity (232 μM/l and 781 μM/l, respectively).

The same tendency was oberves for betanin content (16.3 and 57.8 mg/100 mg) and for total polyphenol quantity (37.5 and 85.5 mg/100 g, respectively).

Varieties of higher betanin and polyphenol contents have higher total antioxidant values and so they are more valuable from nutrition physiological point of view.

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