Antioxidant Activity of Red Beet Juices Obtained after Microwave and Thermal Pretreatments

Anton SLAVOV[1], Vasil KARAGYOZOV[2], Petko DENEV[3], Maria KRATCHANOVA[3] and Christo KRATCHANOV[3]

[1]Department of Organic Cchemistry and [2]Department of Canning Technology, Technological Faculty, University of Food Technologies, Plovdiv, Bulgaria, [3]Laboratory of Biologically Active Substances, Bulgarian Academy of Sciences, Plovdiv, Bulgaria

Abstract

Slavov A., Karagyozov V., Denev P., Kratchanova M., Kratchanov C. (2013): **Antioxidant activity of red beet juices obtained after microwave and thermal pretreatments**. Czech J. Food Sci., **31**: 139–147.

Pressed juices and water extracts from untreated, microwave pretreated, and thermally treated red beet were obtained. The highest total betalain content – 606.34 mg/100 g dry matter (DM) was found in pressed juice obtained after microwave pretreatment. The individual betalains in the pressed juice from untreated red beet were tentatively determined by HPLC. The most abundant betalain pigments were betanin (312.47 mg/100 g DM), vulgaxanthin I (104.08 mg/100 g DM), and isobetanin (71.28 mg/100 g DM). The antioxidant activity of the pressed juices and extracts was determined. The highest antioxidant activity – 10832.4 μmol TE/l was found in the pressed juice obtained from microwave pretreated red beet. The possibility of obtaining mixed fruit-vegetable juices on the basis of red beet juice was investigated. Different variants of mixed beverages using chokeberry, blackberry, blueberry, and black currant were obtained in which the concentration of the red beet pressed juice was 25, 50, and 75%. Their polyphenol contents and antioxidant activity were determined. The highest antioxidant activity revealed mixed pressed juice from red beet and chokeberry.

Keywords: betalains; *Beta vulgaris* L.; ORAC; HORAC; fruit-vegetable juices

Betalains are nitrogenous plant pigments that are characteristic of the order *Caryophyllales*, giving yellow and violet coloration (Strack *et al*. 2003). The interest in betalains has grown since their antiradical activity was characterised (Escribano *et al*. 1998; Kanner *et al*. 2001; Pedreño & Escribano 2001; Číž *et al*. 2010); they are widely used as additives in the food industry because of their natural colorant properties and absence of toxicity (Schwartz *et al*. 1983). Betalains are divided into two groups: betaxanthins and betacyanins, based on their chemical structures. Betaxanthins are condensation products of betalamic acid and amino acids or amines, respectively. Condensation products of betalamic acid and cyclo-Dopa [cyclo-3-(3,4-dihydroxyphenylalanine)] are commonly referred to as betacyanins due to their deep violet colour.

Apart from their use as natural and harmless pigments in the food industry, betalains are important from the medicinal point of view. In a recent study, the antiviral and antimicrobial effects of betalain pigments were reported (Strack *et al*. 2003). Furthermore, the antioxidant properties of betalains were demonstrated in a wide range of assays (Zakharova & Petrova 1998; Gentile *et al*. 2004; Pavlov *et al*. 2005; Číž *et al*. 2010), and it was reported that the enrichment of human low-density lipoproteins by betalains effectively increased the resistance to oxidation (Tesorie-

re *et al*. 2003). In addition, a role of betalain pigments in the chemoprevention against lung and skin cancers was documented (Kapadia *et al*. 1996). It was recently discovered that natural food pigments such as betanin can inhibit the cell proliferation of a variety of human tumour cells (Reddy *et al*. 2005).

The thermal treatment of the food materials is an important method for the preservation, extraction of biologically active substances, and preparation of cooked food products. A disadvantage in the usual thermal treatment of red beet is the betalain instability when exposed to higher temperatures (Havlíková *et al*. 1983; Czapski 1985; Herbach *et al*. 2006). From the technological point of view, regimes of short periods of heating and mild pretreatments are preferred to ensure the preservation of biologically active substances. The microwave pretreatment of the raw material is widely used for the treatment of fresh fruits and vegetables before extraction. It was shown that this pretreatment leads to an increase of the yield of pectic substances from orange peels while the pectic macromolecules are not damaged by the microwave field (Kratchanova *et al*. 2004). It was of interest to investigate the influence of the microwave treatment of red beet on the properties of the obtained juices and extracts and to compare the results with untreated and thermally treated red beet. For this reason, experiments were carried out on microwave pretreatment of raw red beet and the obtained juices and extracts were investigated for their betalain content and antioxidant activity.

The recent trends in the food industry demand the use of natural colour additives. An interesting possibility is mixing extracts containing betalains and anthocyanins that are mutually exclusive in nature (Stintzing *et al*. 2006). In this way, new colour shades and juices having high antioxidant activity and with different colour stabilities can be obtained. However, in that work the antioxidant activity of the obtained mixed juices was not investigated. In our work, we obtained different variants of mixed fruit-vegetable juices and the data on their antioxidant properties wëre investigated.

The aim of the present study was to investigate the antioxidant activity of juices and extracts obtained from non-treated and thermally and microwave pretreated red beet. The possibility to prepare mixed fruit-vegetable beverages on the basis of red beet and berry juices with a pleasant taste and high antioxidant activity was investigated.

MATERIAL AND METHODS

Material and reagents. Red beet roots (*Beta vulgaris* L. cv. Detroit dark red) – dry solids 10.9%, acidity 1.21%, from a a local market in Plovdiv (Bulgaria), was used for the experiments. Before the experiments, the material was kept at 0°C.

Chokeberry (*Aronia melanocarpa*) – dry solids 25.1%, acidity 0.92%, black currant (*Ribes nigrum*) – dry solids 12.4%, acidity 1.38%; blackberry (*Rubus fruticosus*) – dry solids 13.0%, acidity 0.93%, and blueberry (*Vaccinium myrtillus*) – dry solids 13.1%, acidity 1.31%, were supplied at the time of peak production from the region of Rodopi Mountains, Bulgaria. All fruits were delivered at physiologically ripe stage, ready for consumption. Chokeberry (cv. Nero) and black currant (cv. Bentiran) were cultivated whereas blackberry and blueberry were wild-grown. Fruits were immediately frozen and kept at –18°C.

Fluorescein disodium salt, 2,2-azobis-(2-amidino-propane)dihydrochloride (AAPH), 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox), gallic acid, thiobarbituric acid, and Amberlite XAD7 were obtained from Sigma-Aldrich (Steinheim, Germany). Folin-Ciocalteu's phenol reagent was purchased from Merck (Darmstadt, Germany). The betanin standard was purchased from ABCR GmbH and Co. (Karlsruhe, Germany). All other chemicals used were of analytical grade and were purchased from local distributors.

Extraction of pigments from red beet

1.1 *Preparation of pressed juice and water extract from raw untreated red beet* – Peeled red beet (640 g) was cut in pieces of 1 cm, ground using a laboratory blender, and homogenised for 4 min using Polytron (Kinematica GmbH, Olten, Switzerland) to obtain a homogenous mixture. The mixture was pressed through cloth (laboratory rack cloth press (pressure 15 MPa)) for obtaining red beet juice. The residual material was used for further extraction.

The extraction procedure was done four times with different ratios of material to water – 1:4, 1:2, 1:2, and 1:1.5, respectively, depending on the extraction number. The process was carried out at room temperature in the dark (1 h for the first extraction and 0.5 h for the subsequent extractions). After each extraction, the mass was pressed out

(laboratory rack cloth press (pressure 15 MPa)) and finally the extracts were combined.

1.2 *Microwave pretreatment of red beet and extraction* – Peeled red beet (640 g) was cut into pieces of 1 cm. The mass was microwave irradiated 4 times × 3 min at 630 W (after each 3 min irradiation the mass was left to cool to room temperature) and after that was homogenised using first a laboratory blender and then Polytron (Kinematica GmbH, Olten, Switzerland). The obtained mass was pressed through cloth (laboratory rack cloth press (pressure 15 MPa)) to obtain juice. The residue was subjected to the extraction process (as described in 1.1.)

1.3. *Blanching of red beet with water (100°C, 5 min) and extraction* – Peeled red beet was weighed (100 g) and cut in 1 cm pieces. 300 ml water was added and the mixture was heated at 100°C for 5 minutes. After that, the mass was quickly cooled to room temperature (in the dark) and then was homogenised and pressed (as described in 1.1). The residual material was extracted twice with 100 ml water (1 h and 0.5 h for the first and second extractions, respectively).

1.4. *Boiling of red beet (100°C, 15 min) and extraction* – Peeled red beet was weighed (100 g) and cut in 1 cm pieces. 300 ml water was added and the mixture was heated at 100°C for 15 minutes. After that, the mass was quickly cooled to room temperature (in the dark) and the mass was homogenised and pressed (as described in 1.1). The residue was extracted twice with 100 ml water (1 h and 0.5 h for the first and second extractions, respectively).

Preparation of mixed fruit-vegetables juices on the basis of red beet pressed juice

2.1. *Preparation of pressed out juice from red beet* – The red beet was peeled and ground using a laboratory blender and homogenised for 4 min to a homogenous mixture using Polytron (Kinematica GmbH, Olten, Switzerland). The obtained mass was pressed out (laboratory rack cloth press (pressure 15 MPa)) providing pressed out juice.

2.2. *Preparation of pressed out juices from chokeberry, orange, blackberry, blueberry, and black currant* – The raw material was homogenised using a laboratory blender and homogenised for 4 min using Polytron (Kinematica GmbH, Olten, Switzerland) and the mass was pressed (laboratory rack cloth press (pressure 15 MPa)) to obtain pressed out juices.

2.3. *Preparation of mixed juices from red beet, chokeberry, blackberry, blueberry, and black currant* – Twelve mixed fruit-vegetable juices in which the concentration of the pressed juice from red beet was 25%, 50%, or 75% were obtained by mixing juice pressed out from red beet (obtained according to 2.1.) and berry juices obtained according to 2.2.

Spectrophotometric determination of betalains according to Nilson (Socaciu 2008). The absorption at 476, 538, and 600 nm of the juices and extracts was determined. The contents of betacyanins and betaxanthins were determined using the following calculations:

$$x = 1.095(A_{538} - A_{600})$$

$$y = -0.258 \times A_{538} + A_{476} - 0.742 \times A_{600}$$

where:

x – $E_{betanin}$

y – $E_{vulgaxathin\text{-}I}$

Then the concentrations of betacyanins and betaxanthins were determined as:

$$C_{betacyanins} = (x \times R)/(1120 \times 10)\ [mg/ml]$$

$$C_{betaxanthins} = (y \times R)/(750 \times 10)\ [mg/ml]$$

where:

R – dilution factor

1120 – $A^{1\,cm}_{1\%}$ specific absorption of 1% solution betanin in 1 cm

750 – $A^{1\,cm}_{1\%}$ specific absorption of 1% solution vulgaxanthin I in 1 cm

Determination of polyphenol content. Total phenolics were determined according to the method of Singleton and Rossi (1965) with Folin-Ciocalteu's reagent. Gallic acid was employed as the calibration standard and the results were expressed as gallic acid equivalents per liter.

Determination of antioxidant activity of the pressed out juices and the extracts using the ORAC and HORAC methods. Oxygen Radical Antioxidant Capacity assay – ORAC was measured according to the method of Ou *et al.* (2001) with some modifications. The method measures the antioxidant scavenging activity against peroxyl radical induced by AAPH at 37°C. Fluorescein (FL) is used as the fluorescent probe. The loss of fluorescence of FL is the indication of the extent

of damage caused by its reaction with the peroxyl radical. The protective effect of an antioxidant is measured by assessing the area under the fluorescence decay curve (AUC) as compared to that of blank in which no antioxidant is present. The solutions of AAPH, fluorescein, and Trolox were prepared in phosphate buffer (75 mmol/l, pH = 7.4). The samples were diluted with phosphate buffer as well. The reaction mixture (total volume 200 µl) contained FL – (170 µl, final concentration 5.36×10^{-8} mol/l), AAPH – (20 µl, final concentration 51.51 mmol/l), and the sample – 10 µl. FL solution and the sample were incubated at 37°C for 20 min and AAPH (dissolved in 37°C buffer) was then added. The mixture was incubated for 30 s before the initial fluorescence was measured. After that, the fluorescence readings were taken at the end of every cycle after shaking. For the blank, 10 µl of phosphate buffer was used instead of the sample. The antioxidant activity was expressed in Trolox equivalents. Trolox solutions (6.25, 12.5; 25; 50 and 100 µmol/l) were used for constructing the standard curve. One ORAC unit was assigned to the net protection area, provided by the solution of Trolox with the concentration of 1 µmol/l. The final ORAC values were calculated using the regression equation between the Trolox concentration and the net area under the curve. The results were expressed as micromole Trolox equivalents per liter.

HORAC assay – The Hydroxyl Radical Antioxidant Capacity measures the metal-chelating activity of antioxidants in the conditions of Fenton-like reactions employing a Co(II) complex and hence the protecting ability against the hydroxyl radical formation (Ou *et al*. 2002). Hydrogen peroxide solution of 0.55M was prepared in distilled water. 4.6mM Co(II) was prepared as follows: 15.7 mg of $CoF_2 \cdot 4H_2O$ and 20 mg of picolinic acid were dissolved in 20 ml of distilled water. Fluorescein – 170 µl (60nM, final concentration) and 10 µl of sample were incubated at 37°C for 20 min directly in the FLUOstar plate reader. After the incubation, 10 µl H_2O_2 (27.5mM, final concentration) and 10 µl of Co(II) (230µM, final concentration) solutions were subsequently added. The initial fluorescence was measured after which the readings were taken every minute after shaking. For the blank sample, phosphate buffer solution was used. 100, 200, 400, 500, and 600µM gallic acid solutions (in phosphate buffer 75mM, pH = 7.4) were used for constructing the standard curve. The AUC were calculated as in the ORAC assay. The final HORAC values were calculated using the regression equation between the gallic acid concentration and the net area under the curve. One HORAC unit was assigned to the net protection area provided by 1µM gallic acid, and the activity of the sample was expressed as µmol gallic acid equivalents (GAE) per liter. ORAC and HORAC analyses were carried out using a FLUOstar OPTIMA plate reader (BMG LABTECH, Offenburg, Germany), the excitation wavelength of 485 nm and the emission wavelength of 520 nm were used.

Determination of individual betalains by HPLC. The separation and determination of individual betalains by means of HPLC were done using chromatographic system Waters 484 (Waters Millipore, Bedford, USA) using the following conditions: column µ-Bondapak C18 and UV detector R484. Effluent used – CH_3OH:H_2O = 30:70, at 0.3 ml/min, and column temperature 25°C. The detection of the betacyanins and the betaxanthins was done at 540 nm and 480 nm, respectively (Chethana *et al*. 2007), and betanin was used as the reference.

Statistical analysis. All the experiments were done in triplicate and the results are presented as mean values with the standard deviations

RESULTS AND DISCUSSION

Thermal pretreatment of the red beet

The juice obtained through direct pressing of the red beet is rich in betalains and from the nutritive and medicinal points of view is desirable for direct use or incorporation in mixed beverages and other food products. For this reason, we obtained pressed juice from the raw material and then the rest of the material was subjected to additional water extraction to obtain maximum amounts of the pigments and biologically active substances. The influence of the microwave pretreatment and thermal treatment (blanching and boiling) on the yields of juice and betalains was investigated.

The data for betalain content in pressed juice (without thermal pretreatment) are presented in Figure 1. The amount of betacyanins is twice the amount of betaxanthins. The residue after pressing was subjected to fourfold extraction with water and 1010 ml extract in total was obtained. The amount of the extracted betalains was comparable with the amount of betalains obtained after pressing (Figure 1). Almost

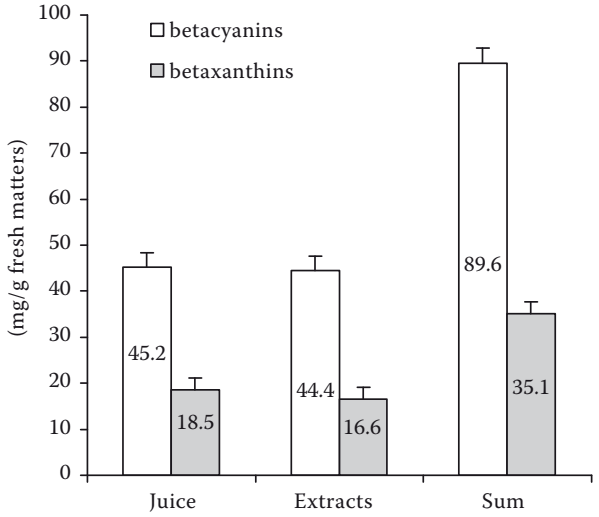

Figure 1. Betalains obtained from raw untreated red beet

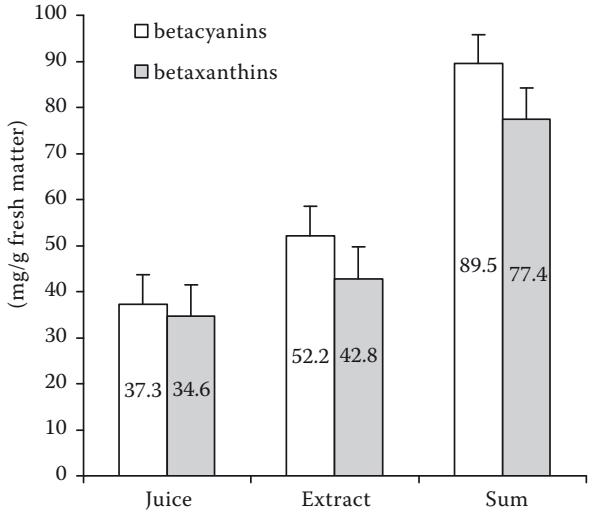

Figure 2. Betalains obtained from microwave pretreated red beet 4 × 3 min, 450 W

the same amount of betalains was left in the residue (less than 30% from raw red beet) after obtaining the pressed juice. Therefore, the content of betalains in the residue after pressing (expressed in percent of their weight) was higher than that of betalains in the pressed juice. In total, 1048 mg of betalains (for 100 g dry matter) were obtained meaning 124.7 mg betalains from 100 g fresh red beet.

In the following experiments the influence of the microwave pretreatment was investigated. Preliminary experiments had shown that higher power of the microwave field and longer times periods increase the temperature of the sample above 60°C which led to the destruction of betalains. The conditions used for the microwave pretreatment – 450 W, 12 min (4 × 3 min) and cooling in the dark after every 3 min, were chosen in order to prevent the increase of the temperature of the microwaved red beet above 50–55°C. After the microwave pretreatment, the sample was quickly cooled to room temperature, homogenised and pressed to obtain pressed juice. The residue was extracted fourfold with water. The amounts of betalains in juice and extract are shown in Figure 2.

The quantities of betacyanins in the obtained pressed juice and extract were lower but the amounts of betaxanthins were almost twice as high – this could be due to the better extraction from the cells. Another possible explanation is the transformation of betalains or partial synthesis of betaxanthins during the microwaving (Schliemann *et al.* 1999; Stintzing *et al.* 1999).

Another widely used treatment of vegetables is the thermal treatment – blanching and cooking. It was of interest to compare these treatments with the previous experiments – with untreated and microwaved red beet. Having in mind the instability of betalains at high temperatures (Herbach *et al.* 2006), we used 5 min for blanching and 15 min for boiling. Since these treatments necessitate the presence of water, only extracts were obtained.

Even the minimal thermal treatment led to a decrease in the yield of the obtained betalains in the extracts (Figure 3). It could be seen also that this was due to the higher decrease of the quantities of betaxanthins which are less stable during the thermal treatments (Herbach *et al.* 2006).

Increasing the period of thermal red beet treatment led to an additional decrease of the betalains content in the extract (Figure 3).

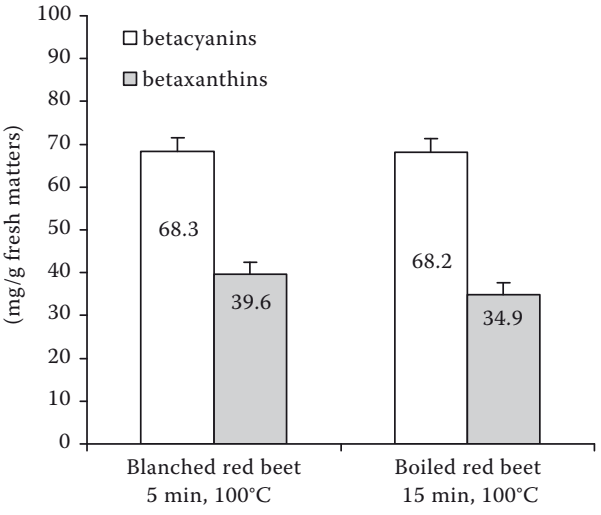

Figure 3. Betalains obtained from thermally treated red beet

Table 1. Content of main betalains in the pressed juice from raw red beet

	Betaxanthins		Betacyanins			
	vulgaxanthin I	vulgaxanthin II	betanin	isobetanin	betanidin	isobetanidin
mg/100 g dry matters	104.1 ± 2.3	57.4 ± 4.9	312.5 ± 12.4	71.3 ± 5.7	18.2 ± 3.4	4.6 ± 0.9
mg/100 g fresh matters	11.4 ± 0.7	6.3 ± 0.9	34.9 ± 1.3	7.8 ± 0.8	2.0 ± 0.3	0.5 ± 0.1

The experiments on the blanching and boiling of the red beet showed that, in order to keep the betalain pigments content at the maximum during the treatments, the heating of the raw materials has to be decreased to minimum or short-time microwave heating has to be used.

Chromatographic analysis

The main betalains in the pressed juice from raw untreated red beet were tentatively determined by HPLC – Table 1. The identified betaxanthins were vulgaxanthin I and II, which have their absorption maximum at 480 nm. Out of the betacyanin, the most abundant was betanin, and in lesser amounts was present isobetanin. Small amounts of the betanin and isobetanin aglycons – betanidin and isobetanidin, respectively, were also observed. Their lower quantities show that the hydrolysis of betacyanins took place in negligible extent.

Antioxidant activity

From the literature is it known that betalain pigments have pronounced antioxidant properties (Pavlov *et al*. 2002; Tesoriere *et al*. 2004). The determination was carried out of the antioxidant activities in the pressed juices and extracts obtained from red beet after different treatments and the results are presented in Table 2.

The highest antioxidant activities were found in the pressed juice and extract obtained from microwave pretreated red beet. This is in contrast with the observed higher amounts of betaxanthins and lesser amounts of betacyanins in the pressed juice and extract from microwave treated red beet (Figure 2) as compared to the quantity of betacyanins and betaxanthins in the pressed juice and extract from the untreated red beet (Figure 1). The possible explanation of these results is the presence of polyphenols (Georgiev *et al*. 2010) in the raw material and it could be concluded that the microwave pretreatment increases the degree of extractability of these substances from the cells.

Preparation of mixed nectars on the basis of pressed juice from raw untreated red beet

Mixing of fruit and vegetable juices and nectars is an interesting possibility for obtaining new beverages with a high antioxidant activity (Stintzing *et al*. 2006). The berry fruits – chokeberry, blackberry, blueberry, black currant etc., are distinctive due to their high antioxidant activity and high contents of anthocyanins and are used for obtaining functional beverages. Also the red beet is a rich source of betalain pigments and has a good antioxidant activity. In the nature, the betalain and anthocyanin pigments are not found together and their mixing may result in beverages having a high antioxidant activity and new colour

Table 2. Antioxidant activities of the pressed juices and extracts obtained from red beet after different treatments

Juice or extract after different treatment	ORAC (μmol TE/l)	Dry matters (%) (refractometrically)	Betalains (mg/ml)
Pressed juice (raw red beet)	9508.7 ± 450.1	6.5 ± 0.2	1.2 ± 0.1
Extract (raw red beet)	278.0 ± 45.2	0.5 ± 0.1	0.1 ± 0.0
Pressed juice (microwaved red beet)	19832.4 ± 780.9	10.5 ± 0.3	1.34 ± 0.2
Extract (microwaved red beet)	855.1 ± 56.7	0.7 ± 0.1	0.1 ± 0.0
Extract from red beet (blanching – 5 min, 100°C)	2358.2 ± 102.3	1.3 ± 0.1	0.3 ± 0.0
Extract from red beet (boiling – 15 min, 100°C)	1639.4 ± 78.9	0.9 ± 0.2	0.2 ± 0.0

Table 3. Characteristics of mixed nectars obtained from raw red beet juices and juices from berries – chokeberry, blackberry, blueberry, and black currant

No	Red beet juice	Chokeberry	Blackberry	Blueberry	Black currant	Polyphenols (mg/l)	ORAC (μmol TE/l)	HORAC (μmol GAE/l)
	(%)							
0	100	–	–	–	–	728.1 ± 6.6	7763.3 ± 380.8	2934.8 ± 271.8
1	–	100	–	–	–	1609.9 ± 35.5	23407.5 ± 774.2	7898.8 ± 619.6
2	–	–	100	–	–	1883.5 ± 37.1	20277.9 ± 1276.8	8382.6 ± 456.3
3	–	–	–	100	–	1202.8 ± 23.9	19194.5 ± 1003.1	5032.8 ± 303.1
4	–	–	–	–	100	1716.2 ± 16.8	20225.7 ± 1080.8	3515.5 ± 337.3
5	25	75	–	–	–	1385.9 ± 35.1	17901.7 ± 724.6	4579.1 ± 322.7
6	50	50	–	–	–	1136.1 ± 29.3	12808.2 ± 620.8	3513.1 ± 348.0
7	25	–	75	–	–	1402.0 ± 21.9	13699.1 ± 773.8	5656.2 ± 429.8
8	50	–	50	–	–	1140.9 ± 37.0	11153.7 ± 853.9	4212.5 ± 197.3
9	25	–	–	75	–	1144.8 ± 10.9	15446.6 ± 755.0	4551.6 ± 149.1
10	50	–	–	50	–	966.9 ± 42.5	12438.0 ± 795.6	4048.0 ± 344.7
11	25	–	–	–	75	1533.5 ± 36.8	15350.3 ± 410.0	4093.0 ± 111.2
12	50	–	–	–	50	1233.8 ± 27.2	10946.0 ± 708.3	3189.3 ± 297.9

schemes for the food industry. These facts give us grounds to develop different variants of mixed fruit-vegetable juices from red beet and berry fruits with the amount of red beet juice of 25% or 50%. A variant in which 75% red beet juice was added was initially developed but after the determination of antioxidant activity in the obtained product it was decided not to use furthermore this concentration of red beet juice. Also, using higher concentrations of red beet juice gave a rise to the "earthy" smell of the obtained mixed red beet – berry beverages. Although the highest antioxidant activity was shown by red beet juice obtained after preliminary microwave treatment of the raw red beet, the beverages made with it showed certain instability with time (mainly due to changes in the colour of the product).

In Table 3 we present the data for polyphenol content and antioxidant activity of the obtained mixed juices.

ORAC method is related to the radical-scavenging activity of antioxidants whereas HORAC method measures the metal-chelating activity of antioxidants in the conditions of Fenton-like reactions and hence the protecting ability against the formation of hydroxyl radical. By means of these two methods, we obtained better information on the antioxidant activity of the mixtures. From one point of view their ability is measured to prevent the radical formation, and from the other one their ability to neutralise the radicals formed.

From the obtained mixed juices, the highest polyphenol contents were shown by sample 5 (red beet with chokeberry), sample 7 (red beet with blackberry), and sample 11 (red beet with black currant) in which the ratio of red beet pressed juice to "berry" juice was 25:75. An interesting observation was that the beverage with the highest polyphenol content (sample 11) did not show the highest antioxidant activity. This was probably due to a different polyphenol profile and the observation that the individual polyphenols have different antioxidant activities (Ou *et al.* 2001). As concerns the antioxidant activity measured by the ORAC test, sample 5 again (red beet juice: chokeberry juice = 25:75) showed the highest antioxidant activity. This juice was also distinctive due to its high antioxidant activity determined by the HORAC method. The highest antioxidant activity of the mixed juices measured by HORAC method was shown by sample 7 (red beet juice:blackberry juice = 25:75).

The obtained mixed nectars were distinctive by their pleasant taste and aroma and natural red colour due to the presence of betalains and antocyanins.

CONCLUSIONS

The experiments aimed at obtaining beverages from red beet subjected to boiling (100°C, 15 min) and blanching (100°C, 5 min) have shown

that the betalain content (both betacyanins and betaxanthins) and antioxidant activity were decreased in comparison with non-treated red beet juice. An alternative pretreatment for obtaining juices and extracts with a higher betalain content and a higher antioxidant activity is mild processing such as microwave irradiation. The analysis of the samples obtained after the microwave treatment of red beet has shown that the antioxidant activity of the juice increased significantly which was probably the result of a better extraction of polyphenols. The betacyanin content was lower compared to the untreated red beet juices but the amount of betaxanthins was doubled. We assume that this increase could be due to the synthesis of betaxanthins during the microwave pretreatment as already observed in controlled experiments on betaxanthins biosynthesis. Due to its high content of betalain pigments and intense red colour, the juice (or red beet itself) is a suitable additive for obtaining vegetable or mixed fruit-vegetable products with natural colour and functionality. Different mixed beverages on the basis of pressed juice from red beet and pressed juices from chokeberry, blackberry, blueberry, and black currant were obtained. The berry fruits juices are distinctive for their high antioxidant activity and pleasant taste. The obtained mixed beverages have a higher antioxidant activity compared with the red beet pressed juice. The highest antioxidant activity measured by the ORAC method was shown by mixed juice red beet-chokeberry (25:75), and measured by the HORAC method by mixed juice red beet-blackberry (25:75).

References

Chethana S., Nayak C.A., Raghavarao K.S.M.S. (2007): Aqueous two phase extraction for purification and concentration of betalains. Journal of Food Engineering, **81**: 679–687.

Číž M., Čížová H., Denev P., Kratchanova M., Slavov A., Lojek A. (2010): Different methods for control and comparison of the antioxidant properties of vegetables. Food Control, **21**: 518–523.

Czapski J. (1985): The effect of heating conditions on losses and regeneration of betacyanins. Zeitschrift für Untersuchung der Lebensmittel, **180**: 21–25.

Escribano J., Pedrēno M.A., García-Carmona F., Muñoz R. (1998): Characterization of the antiradical activity of betalains from *Beta vulgaris* L. roots. Phytochemical Analysis, **9**: 124–127.

Gentile C., Tessoriere L., Allegra M., Livrea M.A., Alessio P.D. (2004): Antioxidant betalains from cactus pear (*O. ficus-indica*) inhibit endothelial ICAM-1 expression. Annals of the New York Academy of Sciences, **1028**: 481–486.

Georgiev V., Weber J., Kneschke E., Denev P., Bley T., Pavlov A. (2010): Antioxidant activity and phenolic content of betalain extracts from intact plants and hairy root cultures of the red beetroot *Beta vulgaris* cv. Detroit dark red. Plant Foods for Human Nutrition, **65**: 105–111.

Havlíková L., Míková K., Kyzlink V. (1983): Heat stability of betacyanins. Zeitschrift für Untersuchung der Lebensmittel, **177**: 247–250.

Herbach K., Stintzing F., Reinhold C. (2006): Betalain stability and degradation – structural and chromatic aspects. Journal of Food Science, **71**: 41–50.

Kanner J., Harel S., Granit R. (2001): Betalains, a new class of dietary cationized antioxidants. Journal of Agricultural and Food Chemistry, **49**: 5178–5185.

Kapadia G., Tokuda H., Konoshima T., Nishino H. (1996): Chemoprevention of lung and skin cancer by *Beta vulgaris* (beet) root extract. Cancer Letters, **100**: 211–214.

Kratchanova M., Pavlova E., Panchev I. (2004): The effect of microwave heating of fresh orange peels on the tissue and quality of extracted pectin. Carbohydrate Polymers, **56**: 181–185.

Ou B., Hampsh-Woodill M., Prior R.L. (2001): Development and validation of an improved oxygen radical absorbance capacity assay using fluorescein as the fluorescent probe. Journal of Agricultural and Food Chemistry, **49**: 4619–4626.

Ou B., Hampsch-Woodill M., Flanagan J., Deemer E., Prior R.L., Huang D. (2002): Novel fluorometric assay for hydroxyl radical prevention capacity using fluorescein as the probe. Journal of Agricultural and Food Chemistry, **50**: 2772–2777.

Pavlov A., Kovatcheva P., Georgiev V., Koleva I., Ilieva M. (2002): Biosynthesis and radical scavenging activity of betalains during the cultivation of red beet (*Beta vulgaris*) hairy root cultures. Zeitschrift für Naturforschung, **57c**: 640–644.

Pavlov A., Kovatcheva P., Tuneva D., Ilieva M., Bley T. (2005): Radical scavenging activity and stability of betalains from *Beta vulgaris* hairy root culture in simulated conditions of human gastrointestinal tract. Plant Foods for Human Nutrition, **60**: 43–47.

Pedreño M.A., Escribano J. (2001): Correlation between antiradical activity and stability of betanine from *Beta vulgaris* L. roots under different pH, temperature and light conditions. Journal of the Science of Food and Agriculture, **81**: 627–631.

Reddy K.M., Ruby L., Lindo A., Nair G.M. (2005): Relative inhibition of lipid peroxidation, cyclooxygenase en-

zymes and human tumor cell proliferation by natural food colors. Journal of Agricultural and Food Chemistry, **53**: 9268–9273.

Schliemann W., Kobayashi N., Strack D. (1999): The decisive step in betaxanthin biosynthesis is a spontaneous reaction. Plant Physiology, **119**: 1217–1232.

Schwartz S.J., von Elbe J.H., Pariza M.W., Goldsworthy T., Pilot H.C. (1983): Inability of red beet betalain pigments to initiate or promote hepatocarcinogenesis. Food and Chemical Toxicology, **21**: 531–535.

Singleton V., Rossi J. (1965): Colorimetry of total phenolic with phosphomolibdiphosphotungstic acid reagents. American Journal of Enology and Viticulture, **16**: 144–158.

Socaciu C. (2008): Food Colorants. Chemical and Functional Properties. CRC Press, New York.

Stintzing F., Schieber A., Carle R. (1999): Amino acid composition and betaxanthin formation in fruits from *Opuntia ficus-indica*. Planta Medica, **65**: 632–635.

Stintzing F., Trichterborn J., Carle R. (2006): Characterisation of anthocyanin-betalain mixtures for food colouring by chromatic and HPLC-DAD-MS analyses. Food Chemistry, **94**: 296–309.

Strack D., Vogt T., Schliemann W. (2003): Recent advances in betalain research. Phytochemistry, **62**: 247–269.

Tesoriere L., Butera D., D'Arpa D., Di Gaudio F., Allegra M., Gentile C., Livrea M.A. (2003): Increased resistance to oxidation of betalain-enriched human low density lipoproteins. Free Radical Research, **37**: 689–696.

Tesoriere L., Allegra M., Butera D., Livrea M. (2004): Absorption, excretion and distribution of dietary antioxidant betalains in LDLs: potential health effects of betalains in humans. American Journal of Clinical Nutrition, **80**: 941–945.

Zakharova N., Petrova T. (1998): Relationships between the structure and antioxidant activity of certain betalains. Applied Biochemistry and Microbiology, **34**: 182–185.

Received for publication February 10, 2012
Accepted after corrections July 7, 2012

Corresponding author:

Dr Anton Slavov, University of Food Technologies, Technological Faculty, Department of Organic Chemistry, 26 Maritza Blvd, Plovdiv 4002, Bulgaria; E-mail: antons@uni-plovdiv.bg