Relationship Between Antioxidant Activities and Red Tablebeet Root Pigments with A Potential Application as a Natural Juice Blend

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ABSTRACT

The antioxidant activities of red tablebeet roots were invesgtiaged using DPPH-ESR system and conjugated diene method. The obtained data indicated an excellent antioxidant and antiradical activities against DPPH and sunflower oil, respectively. The stability of tablebeet pigments against two extreme pH values (3.5 and 8.5) as well as different alevated temperature (30, 50, 75 and 100°C) revealed an excellent free radical scavenging activity even at 100°C. This unique characteristic suggested that red tablebeet juice could be blended with other juices that contained high amount of anthocyanins to substitute the losses that may occur in anthocyanins during pasteurization.

Key Words: red tablebeet root pigments, DPPH, ESR free radical, scavenging activity, conjugated diene, juice blends

INTRODUCTION

Red tablebeet (*Beta vulgaris*) is considered to be a potential source of valuable water-soluble nitrogenous pigments called betalains. These betalains composed of two main groups, the red betacyanins and the yellow betaxanthins (Pavlov *et al.*, 2002). Recently, Kohen & Shalbouh (1994) found that these betalains are cationized compounds, their affinity for membranes may improve their activity.

Kanner *et al.* (2001) emphasized on the strong antioxidant effects of betacyanins in model systems of lipid peroxidations catalyzed by cytochrome C, $H_2O_2^-$ activated metmyoglobin iron redox cycle and lipoxygenase, therfore red tablebeet juice and other red tablebeet products could be used regularly in the diet to provide protection against certain oxidative stress-related disorders in human.

Despite the relative lack of stability of betaline pigments as compared to synthetic food colouring, they are widely used in food products. Delgado-Varags *et al.* (2000) listed numerous products containing betanin food coulourings. They emphasized on the importance of betalain pigments which are particularly suitable for use in food products with a short shelf-life, these products have been produced with minimum heat treatment and then are packaged in a dry state under reduced levels of oxyen and humidity.

Consequently, the present study was carried out to emphasize on the importance of red beet pigments and their application as a food colouring agents to be added to different fruit juice preparations. Kinetic studies were also implemented to find out the possible correlation that may taken place between the red beet pigments and their antioxidative properties measured both as antioxidant and antiradical scavenging activities.

MATERIALS AND METHODS

Materials

Red tablebeet (*Beta vulgaris* L) roots were purchased from the local market at Giza governorate. As soon as they were received, the red tablebeet roots were washed and the pigments were extracted.

Methods:

Red beet pigments extraction

Methanolic extracts were prepared as described by Pavlov *et al.* (2002). Fresh hairy roots were homogenized in 50% ethanol (1g fresh weight/10 ml) three times. The homogenate was centrifuged at (10.000 XG for 10 min). and the resultant supernatants were used for measuring the free radical scavenging by electron spin resonance (ESR) and following up the changes that occurred in the structure by using Raman spectroscopy technique.

Water extracts were also prepared as indicated by Pedreno & Escribano (2001). Root slices (70g) were homogenized at 140 mμ in either phosphate

buffer (pH:8.5) or 50mμ in acetate buffer (pH 3.5) and these solutions were centrifuged at 14.000 XG for 20 min and the obtained supernatant were used for measuring the visible spectra of the beet pigments; their quantifications and for determing the antioxidant activity by conjugated diene method.

FT-Raman spectroscopy

The FT-Raman spectra of the previously mentioned samples were obtained using Nicolet 870 spectrometer with the Nicolt Raman moudule 32B (Madison, Wi, USA) and Ex laser source operated at 1064 nm with a power of 0.7w. The samples were used without further preparation in an NMR glass tube. The calibration was carried out by two possible means, 1st mean by internal calibration using the He-Ne laser beam and the 2nd mean by external calibration using polystryene reference sample. Spectra were obtained in Raman shift range (cm^{-1}) between 200 and 4000 cm^{-1}. The system was operated using omnic 5.1 software and the experiments were replicated three times.

Measurement of the antioxidative properties

Antioxidant activity

The antioxidant activity was measured by applying the conjugated diene method using pure sunflower oil as described by Lingnert *et al.* (1979). The A_{234} was taken as an indication of the course of oxidation using untreated table beet as a control assuming it has 100% activity.

Each extract (0.5-25 mg ml^{-1}) in methanol (100 ml) was mixed with 2 ml of pure sunflower oil adjusted to pH 6.5 in test tubes and placed in darkness at 37°C to accelerate oxidation. After incubation for 15hr. 6 ml of 60% methanol in deionized water was added and the absorbance of the mixture was measured at 234nm against a blank (60% methanol).

Scavenging effect on 2, 2-diphenyl 1-picryl hydrolazyl radicals (DPPH) using ESR technique:

Extracts of the studied samples were prepared as described previously. Four ml were mixed with one ml of methanolic solution containing DPPH 0.2 μ as described by Brunet el al. (2005). The resultant spectra were recorded on an ESR electron spin Bruker-Alex-Sys5000 operated at X-band frequency. The ESR spectrometer set at the following conditions 0.01 field modulation amplitude. The anti-radical activity (AA%) is defined as

$$AA\% = \frac{100.\ (Ho-Hs)}{Ho} \qquad \ldots\ldots\ldots\ldots(1)$$

where :

Ho: Highest of the second peak in the ESR spectrum of DPPH free radical of the blank.

Hs: Highest of the second peak in the ESR spectrum when the extract was added to DPPH.

Determination of tablebeet total pigments

The total betalain pigment contents of beets were measured as sum of betacyanin and betaxanthin concentration according to the method described by Jiratanan & Lin (2004) using the following expression.

$$C=A/\alpha B \qquad \ldots\ldots\ldots\ldots\ldots\ldots..(2)$$

Where:

C: Percent concentration

A: absorbance

α: Molecular absorptivity constant

B: 1cm

Betacyanin has an α of 1120 cm^{-1} % at 535 nm

Organoleptic evaluation

Fresh beet juices were blended with different juices (Guava, pomegranate and red grapes) ; using the following proportions; (1:1, 1:2 and 1:3). These blends were introduced to twenty panelists from the staff members of Food Technology Dept. These samples were evaluated for colour, taste, flavour and acceptability, 10 means highest score where zero means the lowest score.

RESULTS AND DISSCUSION

Visible and Raman spectra of red tablebeet pigments

Data in Figs (1) and (2) show the visible spectra of the betanin solutions at two pH values, 3.5 and 8.5 and at different temperatures 35, 50, 75 and 100°C. Data indicated that there were no marked changes in the shape of visible spectra between the two suggested pH. It seems that temperature effect was obviously indicated rather than the influence of pH. Escribano *et al.* (1998) found that betanin was strongly influenced by pH, this pigment showed greater antiradical activity at neutral and basic pH values than at acidic levels. These results are in agreement with data given by Pedreno & Escribano (2001).

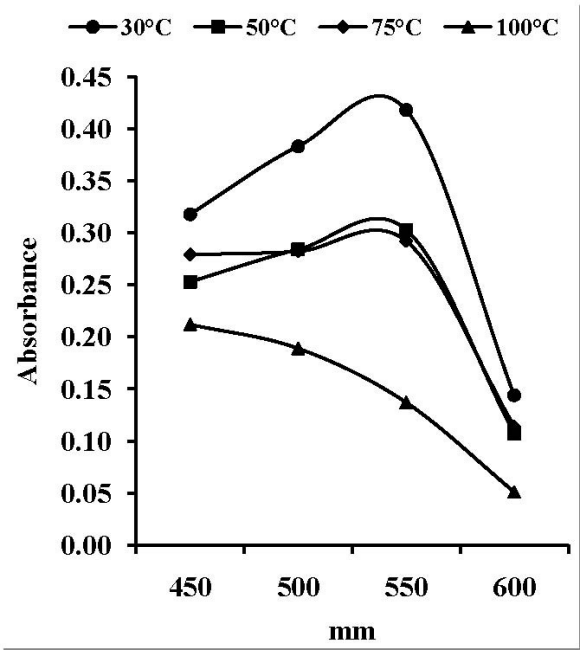

Fig. 1: Visible spectra of red beet root water extracts at pH 8.5 and different temperatures after 25 min incubation period

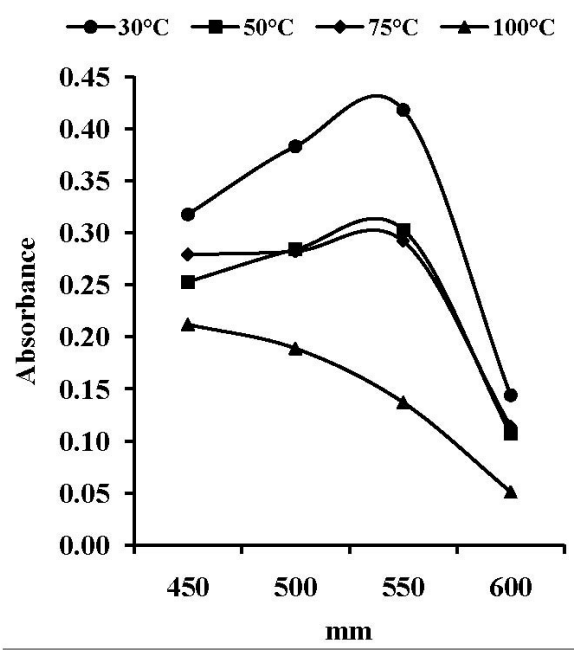

Fig. 2: Visible spectra of red beet root water extracts at pH 3.5 and different temperatures after 25 min incubation period

Raman spectroscopy were also used in the present study to elucidate the structure. Due to the strong electron-photon coupling which occurred in the betanins; two bands were noticed (Fig. 3). The first band was in the range (1400–1600 cm^{-1}). This band could be attributed to the changes that occurred in the double bonds (Withnall *et al.*, 2003) as a function of both temperature and pH. These bands also showed a shift twoards lower band at (1466 cm^{-1}) which indicated effective conjugation length. The second peak (~1025 cm^{-1}) could be attributed to the presence of NH2 groups. The intensity of these bands are getting higher as a function of applying temperature at 100°C and at pH 8.5 and 3.5, respectively. These changes could be related to the instability of the beet pigments and the increament of the degradation products of betanin (Attoe & Von Elbe 1981).

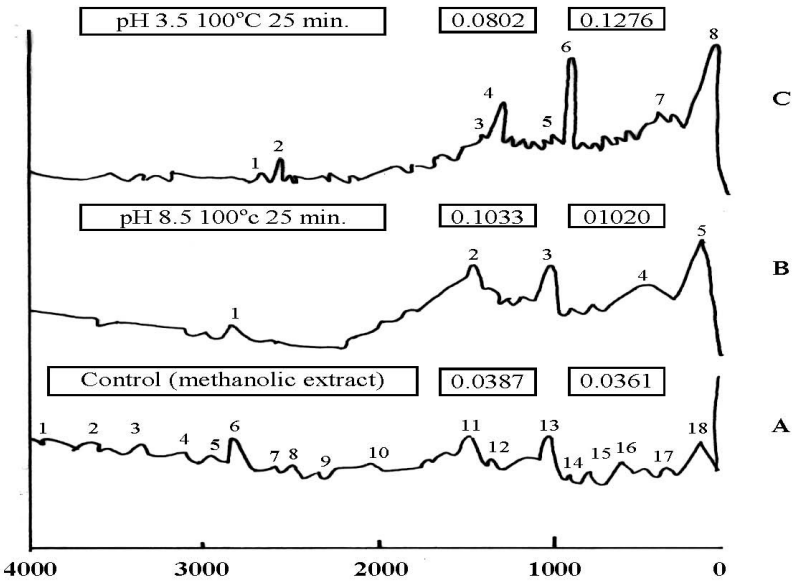

Fig. 3: Changes in the Raman Spectra of red tablebeet root methanolic extracts at different pH values at 100°C

These previous changes that occurred in both the visible and Raman spectra could be related to betanin instability. The removal of oxygen greatly enhanced the heat stability of betanin. The degradation products of betanin that are formed have been identified as betalamic acid (BA) and cyclo-Dopa 5-0-B-D glucoside "CDG" (Schwartz & Van Elbe 1983). The influence of pH on these two previously mentioned degradation products were studied, it was found that BA was most stable at higher pH while (CDG) was most stable at lower pH; (Huang & Von Elbe 1985).

Antioxidant activities of red table beet roots

The antioxidant activities of red tablebeet against DPPH free radical are shown in Fig (4). The antiradical activity at (pH 3.5) was higher than its corresponding at pH 8.5 at 100oC. They were 94.74 and 87.26%, respectively. This observation could be related to GDC which showed high stability at acidic pH. Meanwhile at alkaline pH, the antiradical activity was only attributed to the presence of betanin and not to the degradation products (Pedreno & Escribano 2001). Alkaline conditions brought about aldimine bond hydrolysis; while acidification was found to induce recondensation of betalamic acid and the amino compounds (betaxanthins) or CDG as previously desribed by Schwartz & Von Elbe (1983).

Therefore thermal processing has no impact on the beet antioxidant activity. The loss of pigmentation does not necessarily translate to the loss in antioxidant activity (Savolainen & Kunsi 1978). The obtained ESR signal of the tablebeet root extract, supported this idea, since most of these signals vinashes, indicating strong scavenging activity of DPPH.

The reduction-oxidation cycle imparted by various phytochemicals already embedded in the beet matrix may still preserve the antioxidant activity of betalains even though beet pigments were reduced in the thermal processing. Data in Fig.(5) show the beet antioxidnat activities measured by the conjugated diene method against pure sunflower oil. It seems that the obtained data were also affected by the changes that occurred in the suggested pH values and temperatures ($P<0.01$).

The same figure also revealed that there was a sharp decline in the antioxidant activity (AOA) as a function of temperture increament from 30°C to 50°C followed by a relative stability in the AOA activity at the rest of the studied temperatures. It is suggested that the degradated betanin is partially regenerated. This phenomena is partially affected by the product availability and the buffer used; (Gzapski 1985).

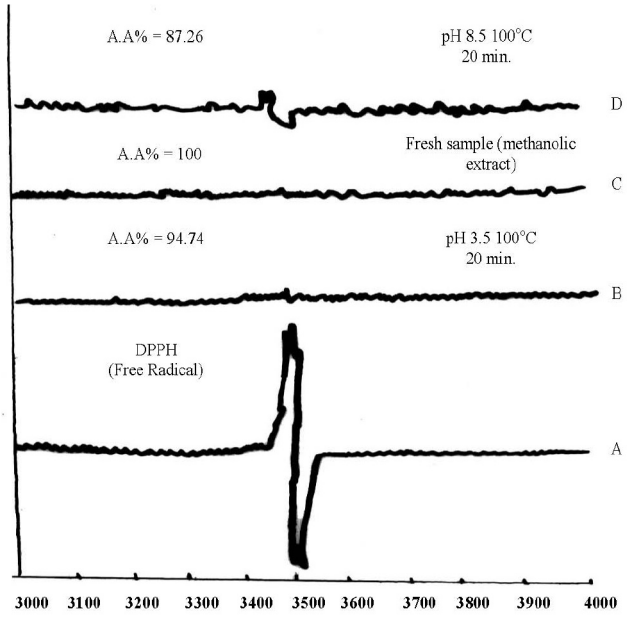

Fig. 4: Changes in the ESR escavenging activity of red tablebeet methanolic extracts as a function of applying different pH values

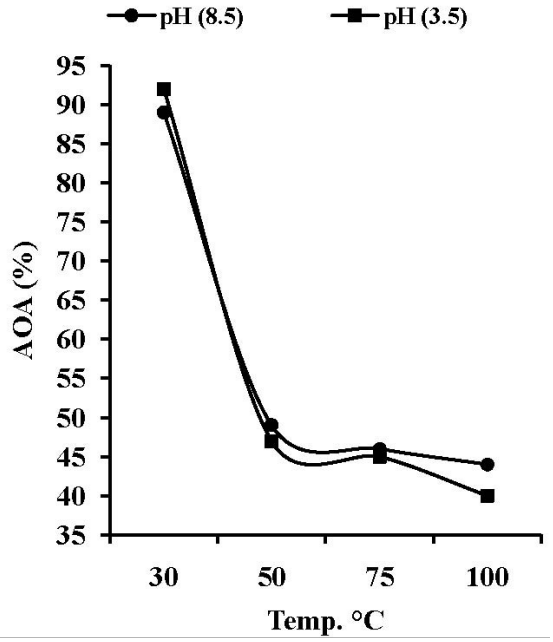

Fig. 5: Changes in the conjugated diene antioxidant activity (AOA%) of beetroot extract as a function of different temperatures and pH values

Beet Pigments

Table (1) shows the changes that occurred in both betaxanthins and betacyanins (mg pigment $100g^{-1}$ beet). The data indicate that both pH values and the suggested temperatures have a significant effect on total pigment values ($P<0.01$). These results are in accordance with those described by Jiratanan & Liu (2004). Also these data indicate that there was a strong correlation coefficients between the loss that occurred in total pigments and the alleviation in temperature degrees as a function of both pH values at 3.5 ($R^2=0.8522$ $P<0.05$) and 8.5 ($R^2=0.9129$ $P<0.01$).

Organoleptic evaluation

Due to the instability of anthocyanins at pH values $\simeq$ 3 (Stintzing & Cark 2004). Betalains are the natural pigments of low acid foods, since betalains can effectively be stabilized by ascorbic acid which on the other hand is known to facilitate anthocyanin degradation (Shenoy 1993). Therefore, using betalains instead of anthocyanins for colouring juices which have high vitamin C content or vitamin-supplemented may be of particular interest. Accordingly, trials were carried out to obtain the most appropriate ratios when fresh beet juice was blended with different fruits rich in anthocyanins like red grapes or pomegranate in addition to guava juices. Juice samples were scored for their organoleptic properties and the points given by the panelists are illustrated in Table (2). The results show that the most appropriate ratios were 1:1 for guava juice and 1:3 for both of pomegranate and red grape juices.

As indicated by Moshammer *et al.* (2005), betalains can cover a broad colour plateau ranging from yellow to purple which may be accomplished by targeted blending of juices or pigment preparation containing betaxanthins and betacyanins, respectively.

Table 1: Influence of pH and temperatures on the pigment contents of red tablebeet measured as betaxanthin, betacyanin and total pigments (mg.100g beet)$^{-1}$

pH	Temp °C	Betaxanthins	Betacyanin	Total pigments
3.5	30	56.31	40.35	96.66
8.5	30	46.69	29.80	76.49
3.5	50	44.31	29.11	73.42
8.5	50	41.07	29.80	70.87
3.5	75	42.46	27.94	70.40
8.5	75	40.00	26.34	66.34
3.5	100	37.23	23.15	60.38
3.5	100	34.44	14.54	48.98

F* = 19.063 P < 0.01

CONCLUSION

The present work was carried out to reveal the importance of blending fresh beet juice with other acidic juices like pomegranate, red grapes or guava. The free radical scavenging activity was stable even at high temperatures (100°C). The losses of red pigments in tablebeet does not necessarily indicate the loss of antioxidant activity.

Table 2: Organoleptic evaluation of fresh beet juice blended with other juices

Blending Ratios	Colour	Aroma	Taste	Acceptability	*P*	F
Guava juice						
1:1	8.0±0.1	7.0±0.20	8.0±0.1	32.0	0.010	7.53
1:2	6.0±0.3	5.2±0.20	6.0±0.3	17.6		
1:3	4.2±05	3.2±0.40	7.0±0.5	12.4		
Pomegranate juice						
1:1	3.0±0.05	6.0±0.12	4.5±0.2	13.5		
1:2	5.0±0.1	7.0±0.14	5.1±0.3	17.5	0.011	7.54
1:3	8.0±0.2	9.0±0.12	7.5±0.2	24.5		
Red grape juice						
1:1	2.5±0.02	5.0±0.12	7.5±0.10	15.0		
1:2	4.5±0.04	8.4±0.17	8.9±0.12	21.8	0.011	7.29
1:3	6.7±0.07	8.9±0.19	9.4±0.14	25.0		

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العلاقة بين النشاط المضاد للأكسدة والصبغات الموجوده فى بنجر المائدة الأحمر ودراسة إمكانية إضافته إلى العصائر الطبيعية

منال فتحى سلامة - عزة أنور أمين - هشام أحمد محرم

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تم فى هذا البحث دراسة النشاط المضاد للأكسدة فى بنجر المائدة الأحمر باستخدام المصائد الإليكترونية وطريقة الرابطة المتبادلة. وقد أوضحت نتائج هذا البحث نشاطاً ممتازً بالنسبة لادمصاص الشقوق الحرة والنشاط المضاد للأكسدة باستخدام DPPH كشق حر وزيت عباد الشمس النقي. وقد تم كذلك دراسة درجة الثبات لصبغات البنجر الحمراء تحت ظروف من قيمتي الأس الهيدروجينى (٥،٣،٥،٨) كذلك عند درجات حرارة مختلفة (٣٠، ٥٠، ٧٥، ١٠٠ درجة مئوية).

وقد أوضحت النتائج أن هناك قدرة امتصاص عالية للشقوق الحرة وهذه الصفة الفريدة مكنت من استخدام صبغة البيتانين فى البنجر بدلاً من الأنثوسانين الموجود طبيعياً فى العصائر والذى يمكن أن يتأثر باستخدام البسترة.