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Research Article

Beta Vulgaris L. Extract: pH Effect on Total Phenolic Content and Antioxidant Properties

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ABSTRACT

Red beet (Beta vulgaris L.) is a rich source of phenolic content including colour pigments and have also high antioxidant capacities. The amounts of their phenolic substances and antioxidant activities change depending on the extraction conditions (pH, time, solvent, etc.). In this work, the coloured water extracts were obtained from red beet at different pH values (pH 4-10) for both an hour and 24 hours. The extracts were evaluated regarding antioxidant activities and total phenolic contents (TPC). The results showed that the total phenolic content of red beet extract in all extraction conditions ranged from 0.55±0.02 to 2.30±0.19 mg GAE/g FW. The highest total phenolic contents (2.30±0.19 mg GAE/g FW) were obtained from red beet at 24 h and pH=4. On the other hand, while the IC_{50} values for DPPH activity of red beet extracts at all pH values and times are between 0.84±0.04 and 5.44±0.75 mg/mL, the IC_{50} values for ABTS activity are between 1.46±0.42 and 3.65± 0.28 mg/mL. The extract obtained from red beet at 24 h and pH=4 exhibited the strongest DPPH activity with the IC_{50} values of 0.84±0.04 mg/mL, it showed the best ABTS activity with the IC_{50} values of 1.46±0.42 mg/mL at 24 h and pH=4.

1. Introduction

Naturally occurring colour pigments are important determinants of quality attributes in fresh fruits and vegetables [1]. Colourful fruits and vegetables have become very popular today due to the vitamins, minerals and bioactive substances they contain, and they are recommended to be consumed daily [2]. Consumption of fruits and vegetables, as well as nutrition, should be stored in a value that should be taken into account due to the evaluation of their importance in human health [3].

Many types of vegetables are grown in Türkiye, and red beet (*Beta vulgaris* L.) has an important place among these vegetables [4]. Red beet is grown and widely consumed all over the world [5]. All types of beet leaves can be eaten raw or cooked. It is rich in nutrients such as vitamins A, K, C, protein, fibre, calcium, manganese, magnesium, potassium, copper, iron, zinc, phosphorus [6]. Red beet helps to lower high blood pressure, cardiovascular diseases, raises haemoglobin. In addition, it reduces obesity, lowers blood sugar, improves skin, hair, eye, bone and muscle health, and is also a powerful antioxidant [7]. Red beet contains many bioactive compounds (flavonoids, carotenoids, betalain, ascorbic acid, polyphenols) that are effective on health [8].

In nature, betalains are commonly found in roots, fruits, and flowers [9]. They are used to add nutritional value to food, change colour or give colour to food. The most common source of betalain is beetroot [10]. Betalains are water-soluble and nitrogen-containing-coloured pigments known as betalaminic acid derivatives [11].

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The molecular structures of the compounds that give colour to the red beet is given in Figure 1.

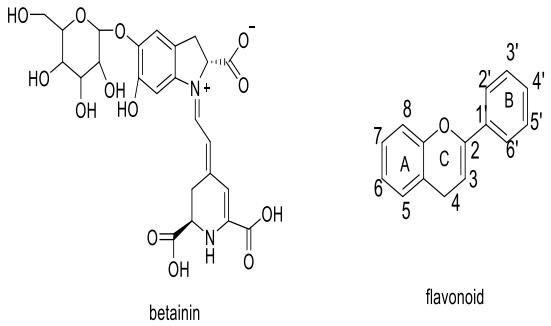

Figure 1. Molecular structure of some colour pigments

In this study, total phenolic compounds were extracted from red beet at different pH/different time intervals. The extraction time and pH effects on total phenolic content and ABTS and DPPH activities as antioxidant properties were investigated.

2. Materials and Methods

2.1. Materials

All used chemicals were purchased from Sigma Aldrich and Merck. Red beet, grown in Türkiye, were sourced from the local market.

2.2. Extraction

Red beet sample was dissected after cleaning. 10 g sample was extracted with distilled water. The extraction conditions of the samples were adjusted to different pH values (pH 4-10). pH treatment was carried out with citric acid and sodium carbonate (Na_2CO_3). The extracts were stored in jars in dark until their analysis.

2.3. Total Phenolic Content (TPC)

Folin–Ciocalteu reagent was used when determining total phenolic content [12]. Stock extracts were mixed with diluted Folin-Ciocalteu reagent. This solution was incubated at 25°C for 3 min. and mixed with 1 mL of Na_2CO_3. The absorbance value was measured at 765 nm after 60 min. of incubation in the dark. Drawing a standard curve with gallic acid. The total amount of phenolic compounds was calculated and expressed as mg GAE (Gallic Acid Equivalent) /g FW (fresh weight).

2.4. DPPH Radical Scavenging Activity

DPPH activity of the extract was determined using Sönmez et al. as a reference [13]. Samples at different concentrations were prepared from extracts with different pH values. 3 mL of DPPH solution was mixed with these different concentrations of the extracts. This thoroughly mixed solution was incubated for 30 minutes. After the absorbance of these solutions was measured at 517 nm. IC_{50} values were determined by calculating DPPH activity.

$$\%\ \text{DPPH inhibition} = [(Abs_c - Abs_s) / Abs_c]*100 \quad (1)$$

2.5. ABTS Radical scavenging activity

ABTS scavenging activities of the extract was measured according to the method described by Sonmez et al. [14]. ABTS radical solution was generated by mixing 19.2 mg of ABTS and $K_2S_2O_3$. This solution was kept at 25°C and in the dark for one day. The absorbance of this prepared solution was adjusted to be 0.70±0.01 at 734 nm. It was mixed with ABTS prepared with samples of different concentrations. The absorbance of the samples was measured at baseline and after 6 minutes. The measurement was made at a wavelength of 734 nm. The absorbance-concentration graph was drawn. IC_{50} value was calculated as mg/mL.

3. Results and Discussion

The graphical representations of total phenolic contents, DPPH and ABTS activity results are presented in Figures 2-4.

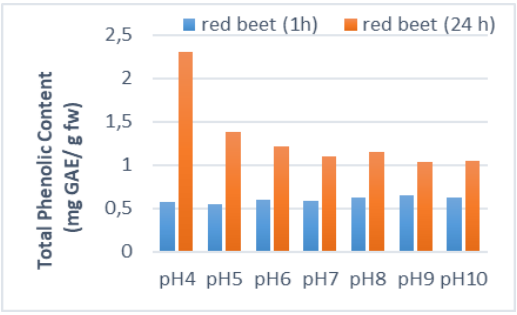

Figure 2. Graphical representation of TPC results for red beet.

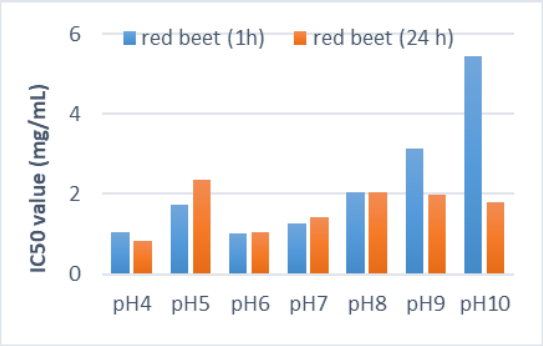

Figure 3. Graphical representation of IC_{50} values of red beet extracts for DPPH activity.

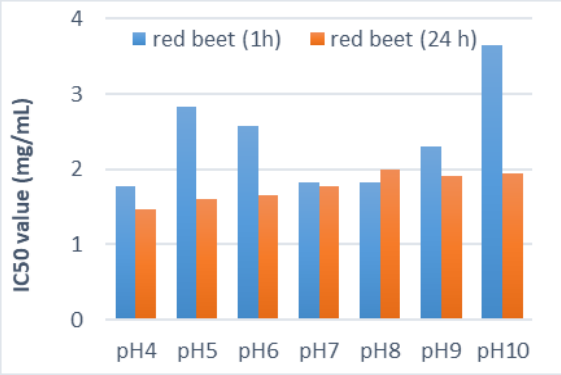

Figure 4. Graphical representation of IC_{50} values of red beet extracts for ABTS activity.

Total phenolic content and antioxidant activity values of red beet is presented in Table 1-2.

The results showed that total phenolic contents (TPC) of red beet extract is between 0.55±0.02 and 0.65±0.06 mg GAE/g FW at pH=4-10 and 1h. The total phenolic content of red beet extract is between 1.03±0.09 and 2.30±0.19 mg GAE/g FW at pH=4-10 and 24h. Red beet extract had the high total phenolic content at 24h and pH=4.
Red beet extracts showed DPPH activity with an IC_{50} value of 1.01± 0.28 - 5.44± 0.75 mg/mL at 1h and with an IC_{50} value of 0.84± 0.28 - 2.37± 0.39 mg/mL at 24h. Among them, red beet extract exhibited the strongest DPPH activity at 24h and pH=4.

Table 1. TPC and DPPH, ABTS activity (IC_{50} values) of red beet extracts obtained for 1h.

pH	Sample	TPC (mg GAE/ g FW)	DPPH (IC_{50}, mg/mL)	ABTS (IC_{50}, mg/mL)
		Extraction time		
		1h	1h	1h
4	red beet	0.57± 0.02	1.06± 0.03	1.78± 0.23
5	red beet	0.55± 0.02	1.73± 0.49	2.83± 0.53
6	red beet	0.60± 0.06	1.01± 0.28	2.57± 0.67
7	red beet	0.59± 0.07	1.25± 0.43	1.82± 0.18
8	red beet	0.63± 0.01	2.05± 0.47	1.82± 0.45
9	red beet	0.65± 0.06	3.13± 1.06	2.30± 0.50
10	red beet	0.62± 0.1	5.44± 0.75	3.65± 0.28

Results are expressed as means ± SD (standard deviation) (n=3).

Table 2. TPC and DPPH, ABTS activity (IC_{50} values) of red beet extracts obtained for 24h.

pH	Sample	TPC (mg GAE/ g FW)	DPPH (IC_{50}, mg/mL)	ABTS (IC_{50}, mg/mL)
		Extraction time		
		24 h	24 h	24 h
4	red beet	2.30± 0.19	0.84± 0.04	1.46± 0.42
5	red beet	1.38± 0.16	2.37± 0.39	1.61± 0.12
6	red beet	1.21± 0.12	1.04± 0.05	1.66± 0.26
7	red beet	1.10± 0.07	1.41± 0.01	1.78± 0.18
8	red beet	1.15± 0.07	2.04± 0.97	1.99± 0.04
9	red beet	1.03± 0.04	1.97± 0.24	1.91± 0.07
10	red beet	1.05± 0.06	1.78± 0.01	1.95± 0.38

Results are expressed as means ± SD (standard deviation) (n=3).

Red beet extracts showed ABTS activity with an IC_{50} value of 1.78± 0.23 - 3.65± 0.28 mg/mL at 1h, and with an IC_{50} value of 1.46± 0.42 - 1.99± 0.04 mg/mL at 24h. Among them, red beet extract exhibited the highest ABTS activity at 24h and pH=4.

4. Conclusion

In conclusion, red beet extracts, obtained different pH values and extraction time, were compared in terms of total phenolic content and antioxidant properties. The results show that the phenolic content of red beet is between 0.55±0.02 and 2.30±0.19 mg GAE/g FW. Moreover, the red beet extracts showed strong antioxidant activity. It was determined that the extraction time is 24h and pH value is 4 for the highest TPC of red beet extract. On the other hand, it was observed that extraction time is 24h, pH values are 4 as the best conditions for DPPH and ABTS activity. According to all these results, it is considered that red beet extracts may be preferred to use for natural colorant additive for various nutrition.

Article Information Form

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Authors' Contribution
The authors who have made substantial contributions to the work reported in the manuscript are:

R.A. and Z.S.: Term, Conception and design of study, Visualization, Writing - Original Draft, Data Curation, Investigation, Formal analysis, Validation. F.S. and M.K.: Supervision, Project administration, Conceptualization, Writing-Original Draft, Data Curation, Investigation, Formal analysis, Validation, Methodology.

The Declaration of Conflict of Interest/ Common Interest
No conflict of interest.

The Declaration of Ethics Committee Approval
This study does not require ethics committee permission or any special permission.

The Declaration of Research and Publication Ethics
The authors of the paper declare that they comply with the scientific, ethical and quotation rules of SAUJS in all processes of the paper and that they do not make any falsification on the data collected. In addition, they declare that Sakarya University Journal of Science and its editorial board have no responsibility for any ethical violations that may be encountered, and that this study has not been evaluated in any academic publication environment other than Sakarya University Journal of Science.

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