RESEARCH ARTICLE

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The effect of pH and extraction time on total phenolic content and antioxidant properties of coloured water extracts from *Brassica Oleracea*

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Abstract

Red cabbage (Brassica oleracea) 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 extract was obtained from red cabbage at different pH values (pH 4-10) for both an hour and 24 hours. The extracts were evaluated regarding total phenolic contents and antioxidant activities. The results showed that the total phenolic content of red cabbage extracts in all extraction conditions ranged from 4.93±0.20 to 7.59±1.22 mg GAE/g fw. The highest total phenolic contents (7.59±1.22 mg GAE/g fw) were obtained from red cabbage at 24 h and pH=6. On the other hand, the red cabbage extracts have high DPPH (IC_{50} values ranged from 0.21±0.06 to 0.94±0.03 mg/mL) and ABTS (IC_{50} values ranged from 0.29±0.01 to 0.46±0.05 mg/mL) activities at all pH values and times. The extract obtained from red cabbage at 1 h and pH=7 exhibited the strongest DPPH activity with the IC_{50} values of 0.21±0.06 mg/mL, it showed the best ABTS activity with the IC_{50} values of 0.29±0.01 mg/mL at 1 h and pH=5 and 8.

Keywords: Red cabbage, pH effect, total phenolic content, DPPH Scavenging, ABTS activity

INTRODUCTION

Coloring substances have different chemical structures. Therefore, it has different physical, chemical and physicochemical properties. Naturally occurring colour pigments are important determinants of quality attributes in fresh fruits and vegetables (Stintzing and Carle, 2004). Colorful 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 (Amao, 2018). Consumption of fruits and vegetables, as well as nutrition, should be stored in a requirement that should be taken into account due to the evaluation of their importance in human health (Ülger et al., 2018).

Nearly 50 types of vegetables are grown in Türkiye, red cabbage (*Brassica oleracea* L.) has an important place among these vegetables (Karl and Koch, 2013). Red cabbage is a seasonal plant, a type of vegetable that is grown as a red/purple leafy autumn vegetable with large, thick and dark leaf layers. The higher the beta-carotene ratio, the darker the colour (Maltas et al., 2017). Red cabbage gives a red colour in acidic environments and dark blue in basic environments, and it is very important for organic structure that these colours are stable at room temperature. This colour is due to the presence of anthocyanins and is used as a natural food colouring (Chigurupati et al., 2002; Chen et al., 2021). Red cabbage contains a number of bioactive substances such as manganese, potassium, magnesium, calcium, iron and vitamins C, E, K and B6, oligosaccharides, anthocyanins,

flavonols and glucosinolates (Wiczkowski et al., 2013). It helps in the prevention of stomach, lung, intestine, heart diseases and strengthens the immune system. It is known to have anticancer effects (Laje et al., 2010). Moreover red cabbage anthocyanins have received much attention from researchers due to their low cost, availability, abundance, and reliable halochromic capacity (Hosseini et al., 2016; Liang et al., 2019).

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

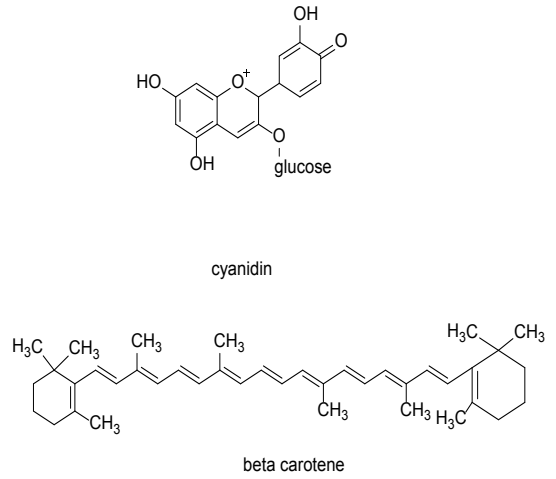

Figure 1. Molecular structure of some colour pigments

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

MATERIALS AND METHODS

Materials

All used chemicals were purchased from Sigma Aldrich and Merck. Red cabbage, grown in Aladağ, Adana (Latitude: 37°32′47″N, Longitude: 35°23′55″E, Türkiye), was sourced from the local market.

Extraction

Red cabbage samples were 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 (0.1 N) and sodium carbonate (Na_2CO_3, 10% (w/v)). The extracts were stored in jars in dark at room temperature until their analysis. The color scale of the extracts at different pH values is given in Figure 2.

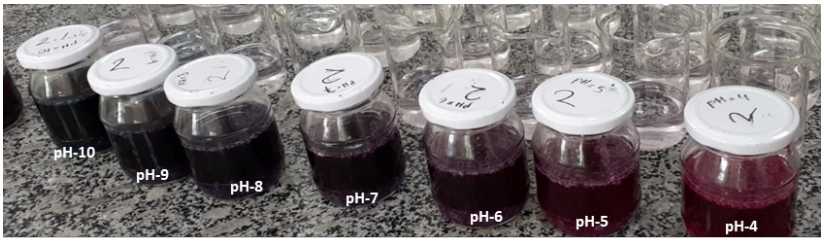

Figure 2. The color scale of the extracts at different pH values.

Total Phenolic Content (TPC)

The total phenolic content of extract was determined by using Folin–Ciocalteu reagent assay (Sonmez and Sahin, 2023). Stock extracts were mixed with Folin-Ciocalteu reagent diluted 1:10 with water. 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. A standard curve with gallic acid was drawn. The total amount of phenolic compounds was calculated and expressed as mg GAE (Gallic Acid Equivalent) /g fw (fresh weight).

DPPH Radical Scavenging Activity

DPPH radical scavenging activity of extract was measured according to the method described by Sonmez et al.

(Sonmez et al., 2019). 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 incubation, absorbance values were determined at 517 nm wavelength. IC_{50} values are calculated by from the graph of %inhibition against the extract concentration.

% DPPH inhibition = $[(Abs_c - Abs_s) / Abs_c]*100$

ABTS Radical Scavenging Activity

ABTS scavenging activities of the extract were measured according to the method described by Sonmez et al. (Sonmez et al., 2022). The solution of ABTS radical was generated by dissolving ABTS and $K_2S_2O_3$ in distilled water. This solution was kept in dark for 18-24 h at room temperature. 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 734 nm 6 min. after mixing. The change in absorption was used for calculation of activity.

Statistical analysis

The data are presented as mean ±SD for triplicate analysis. Statistical evaluations were analysed by Minitab Statistical Software using ANOVA with a 95% confidence interval.

RESULTS AND DISCUSSION

Total phenolic content and antioxidant activity values of red cabbage are presented in Table 1.

The results showed that total phenolic contents (TPC) of red cabbage extract is between 4.93± 0.2 and 6.48± 0.77 mg GAE/g fw at pH=4-10 and 1h. The total phenolic content of red cabbage extract is between 5.64± 0.16 and 7.59± 1.22 mg GAE/g fw at pH=4-10 and 24h. Red cabbage extract had the highest total phenolic content at 24h and pH=6. Generally, increasing extraction time raised the TPC of red cabbage. In the study conducted by Hunaefi (2013) on red cabbage, its total phenolic content was stated as (175.89 ± 15.99 mg GAE.100g fw^{-1}).Moreover the total phenolic content of four common vegetables was determined by Djeussi et al. (2022) and red cabbage was reported as 4.37±0.32 mg of gallic acid/ gram of dried extract. It was observed that all of the total phenolic content measured at different pH and different times were higher than the value reported by Hunaefi et al. and by Djeussi et al.

Red cabbage extracts showed DPPH scavenging activity with an IC_{50} value of 0.21± 0.06 - 0.34± 0.01 mg/mL at 1h and with an IC_{50} value of 0.34±0.01 - 0.94±0.03 mg/mL at 24h. Among them, red cabbage extract exhibited the strongest DPPH scavenging activity at 1h and pH=7. The antioxidant activity of fresh and dried red cabbage was examined by Efendi et al (2022). The antioxidant activity IC_{50} value of fresh and dried red cabbage was reported as 54.317 ppm and 49.464 ppm, respectively. In addition to the antioxidant properties of red cabbage in different solvents were stated by Önder et al. (2020), and IC_{50} values were determined as 63±1.41 µg/mL in the ethenol:water extract and 60±1.41 µg/mL in the methanol extract. The antioxidant effect of the extracts obtained in this study is much stronger than the two reported studies.

Red cabbage extracts showed ABTS activity with an IC_{50} value of 0.29± 0.01 - 0.38± 0.02 mg/mL at 1h, and with an IC_{50} value of 0.33±0.01 - 0.46±0.05 mg/mL at 24 h. Among them, red cabbage extract exhibited the highest ABTS activity at 1h and pH=5 and pH=8.

Table 1. Total phenolic content (TPC), DPPH scavenging activity and ABTS activity (IC_{50} values) of red cabbage extracts obtained for 1h and 24 h.

pH	Sample	TPC (mg GAE/ g fw)		DPPH (IC_{50}, mg/mL)		ABTS (IC_{50}, mg/mL)	
		Extraction time					
		1h	24 h	1h	24 h	1h	24 h
4	red cabbage	6.48±0.77^{a}	7.01±0.24^{a,b}	0.32±0.08^{a}	0.34±0.01^{d}	0.32±0.03^{c}	0.46±0.05^{a}
5	red cabbage	5.66±0.22^{b}	6.99±0.54^{a,b}	0.34±0.01^{a}	0.39±0.06^{c,d}	0.29±0.01^{d}	0.33±0.01^{b}
6	red cabbage	5.37±0.09^{c}	7.59±1.22^{a}	0.26±0.02^{b}	0.46±0.16^{b,c,d}	0.32±0.02^{b,c}	0.38±0.03^{b}
7	red cabbage	5.37±0.14^{c}	5.95±0.42^{c}	0.21±0.06^{c}	0.67±0.14^{a,b,c}	0.32±0.09^{d}	0.35±0.01^{b}
8	red cabbage	5.62±0.40^{b}	6.36±0.20^{c,b}	0.28±0.08^{a,b}	0.72±0.18^{a,b}	0.29±0.11^{d}	0.33±0.01^{b}
9	red cabbage	4.94±0.22^{d}	6.28±0.10^{c,b}	0.25±0.11^{b}	0.94±0.03^{a}	0.34±0.08^{b}	0.34±0.01^{b}
10	red cabbage	4.93±0.20^{d}	5.64±0.16^{c}	0.25±0.06^{b}	0.89±0.83^{a}	0.38±0.02^{a}	0.37±0.02^{b}

Results are expressed as means ± SD (standard deviation) (n=3). a-d Refers the significant differences between the values in the same column ($P < 0.05$).

Generally, red cabbage extract has high TPC and also stronger DPPH and ABTS activities both extraction time and all pH values. The changing extraction time and pH did not linearly affect DPPH and ABTS activities of this extract.

CONCLUSIONS

In conclusion, red cabbage extracts, obtained different pH values and extraction time, were compared in terms of total phenolic content and antioxidant properties. The results showed that red cabbage had a significantly high phenolic content. Moreover, the red cabbage extracts showed strong antioxidant activity. It was determined that the extraction time is 1h and pH value is 6 for the highest TPC of red cabbage extract, while extraction time is 1h and pH value is 7 for the strongest DPPH activity. On the other hand, it was observed that extraction time is 1h, pH values are 5 and 8 as the best conditions for ABTS activity. Total phenolic content, DPPH and ABTS antioxidant activity values of the samples extracted under different pH conditions were found to be statistically significant ($p<0.05$). According to all these results, it is considered that red cabbage extracts may be preferred to use for as natural colorant additive for various nutrition.

COMPLIANCE WITH ETHICAL STANDARDS

Peer-review

Externally peer-reviewed.

Conflict of interest

The authors declare that they have no conflict of interest.

Author contribution

The contribution of the authors to the present study is equal. All the authors read and approved the final manuscript. All the authors verify that the Text, Figures, and Tables are original and that they have not been published before.

Ethical approval

Ethics committee approval is not required.

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Data availability

Not applicable

Consent for publication

Not applicable.

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