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The effect of different cooking methods on the antioxidant activity of wild Swiss chard (*Beta vulgaris* L. var. *cicla*)

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Abstract: This study aimed to investigate the effects of different cooking methods (boiling for 5 min and 8 min, microwave cooking for 3.5 min and 6.5 min, and stir-fry for 5 min and 10 min) on wild chard's antioxidant activity and total phenolic content. It was determined that the total phenolic content [1 552.00 ± 299.82 mg GAE·(100 g)$^{-1}$, GAE – gallic acid equivalents] increased in 3.5 min in the microwave [2 611.33 ± 311.76 mg GAE·(100 g)$^{-1}$] and 5 min in stir-frying [2 434.33 ± 197.75 mg GAE·(100 g)$^{-1}$] compared to the raw chard samples ($P < 0.05$). Two different antioxidant activity determination methods [2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity and ferric reducing antioxidant power (FRAP)] were used in the study. In both methods, it was determined that antioxidant activity decreased in the 10-min application of stir-frying. In contrast, the antioxidant activity increased by applying 3.5 min and 6.5 min in the microwave ($P < 0.05$). As a result, it was found that the antioxidant activity and total phenol content of wild chard changed when the cooking method and time were changed.

Keywords: boiling; microwave; stir-fry; total phenolic content; wild chard

Chard (*Beta vulgaris* L. var. *cicla*) is an important product because it can be used throughout the year and contains plenty of minerals, vitamins and phenolic compounds. Chard leaves and stems are used in many traditional meals and salads (Trifunovic et al. 2015). When the benefits of chard on human health are examined, it is known that it is antioxidant, anti-diabetic and anti-carcinogenic. It is assumed that especially wild plants are a very rich and natural source of antioxidants that contribute to reducing potential risks by scavenging free radicals (Gamba et al. 2021).

Oxidative free radicals are by-products of normal reactions in our bodies. These reactions can include energy production, degradation of lipids, catecholamine response under stress, and inflammatory processes. Free radicals play an important role in developing various pathological conditions such as lipid peroxidation, protein oxidation, DNA (deoxyribonucleic acid) damage and cellular degeneration (Jideani et al. 2021). Antioxidants clean up the free radicals and prevent oxidative damage to proteins, DNA, and lipoproteins (Gamba et al. 2021).

Human health has become extraordinarily important in recent years due to the growing risk factors for various serious diseases. Vegetable and fruit consumption reduces the risk of many chronic diseases. Vegetables provide a variety of phytochemicals that are involved in antioxidant protection (Jideani et al. 2021). Phytochemical compounds reduce the incidence of tumours and increase life expectancy. It also reduces oxidative

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damage and changes apoptosis rates. Many studies are showing an important link between vegetable intake and decreasing risks of cardiovascular disease, different cancers, autoimmune diseases, atherosclerosis, Alzheimer's disease, cataracts, many other degenerative diseases, and ageing (Akbari et al. 2022).

Many vegetables are consumed after being cooked in water or oil media, over a wide temperature range, and under different conditions. Therefore, the heat process becomes crucial as it will directly affect the final composition of vegetables. The heating process can cause a series of changes in texture, colour and structure (Samaniego-Sánchez et al. 2015). Various cooking methods are used in vegetables depending on the temperature. The most commonly used cooking methods are boiling, steaming and stir-frying (Pérez-Burillo et al. 2019). Cooking medium (water or oil) is essential in vegetables because it is thought that besides the concentration of water-soluble compounds may decrease, compounds may increase with the effect of temperature when using an aqueous medium (Natella et al. 2010). When oil is used in cooking vegetables, it is observed in studies that the phytochemical content of vegetables can increase the antioxidant activity due to improvement (Samaniego-Sánchez et al. 2015; Pérez-Burillo et al. 2019). In addition to the cooking methods used, the effect of the processing time is also significant. Therefore, a lengthy cooking procedure may result in the loss of bioactive compounds (Rufián-Henares et al. 2013).

This study investigates the effects of different cooking methods on wild chard's antioxidant activity and total phenolic content.

MATERIAL AND METHODS

Plant material

An average of 1 kg of wild chard samples was collected from the Derepazarı district of Rize in Türkiye. The inedible parts of the vegetables were removed by hand or using a sharp knife, washed with tap water, and dried with a paper towel. After cleaning, an average of 525 g of samples remained. The remaining vegetables were cut into homogeneous, equal small pieces (3 × 3 cm).

For each application, 75 g of chard was weighed. One portion was stored raw at +4 °C until all cooking methods were completed (approximately 30 min). Three different cooking methods with varying cooking conditions and durations were applied to the other samples (Figure 1).

Boiling. 75 g of chard sample was added to 300 mL of tap water, which came to the boiling point (96.7 ± 1.0 °C) in a stainless steel pot with a diameter of 15 cm. Chard samples were cooked for 5 min and 8 min, removed when their time expired, filtered and cooled with ice moulds.

Microwave cooking. Chard samples (75 g) were placed on a glass plate, and 10 mL of water was added. Then, it was cooked in a 600-watt microwave oven (MS23F300EEK; Samsung, Korea) for 3.5 min and 6.5 min. Chard samples were rapidly cooled.

Stir-frying. 10 mL of sunflower oil was added to the wok pans, and the temperature was allowed to reach 180 °C. Then, chard samples (75 g) were added and mixed, and two different cooking times were applied, 5 min and 10 min. The samples were rapidly cooled.

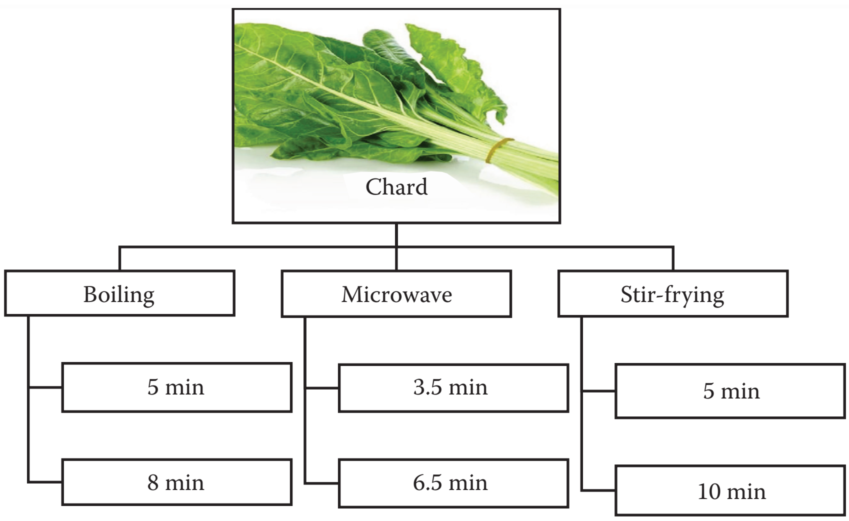

Figure 1. Different cooking methods and times applied to wild chard

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Dry matter determination

Raw and cooked vegetables were homogenised in a blender (LM2421; Moulinex, France) for 2 min. To determine the dry matter content, 3 g of raw or cooked homogenised sample was oven-dried at 70 °C for at least 2 days until a constant weight was reached (Turkmen et al. 2005).

Analytical method

About 1 g of raw and cooked homogenised samples was added to methanol and shaken in a mechanical shaker for 2 h. Mixtures were filtered through Whatman No. 1 filter paper. The filtrates were used to determine both phenolic compounds and antioxidant activity.

Determination of total phenolic content

Total phenolic content was determined using the Folin-Ciocalteu reagent Singleton and Rossi (1965) described. Results were expressed as milligram gallic acid equivalents GAE·$(100\ g)^{-1}$ dry weight.

Determination of antioxidant activity

The antioxidant activities of the samples were determined using FRAP and DPPH methods. The FRAP method is based on reducing the yellow Fe^{3+}-TPTZ complex (TPTZ – 2,4,6-tripyridyl-s-triazine) to the blue Fe^{2+}-TPTZ complex (Benzie and Szeto 1999). Results were expressed as μmol $FeSO_4 \cdot 7H_2O$ equivalents per 100 g of dry matter.

DPPH radical scavenging activity is based on the purple-to-yellow colour change of the DPPH solution when the radical is deactivated by antioxidants (Molyneux 2004). Trolox was used as a standard, and results are given as IC_{50} ($mg \cdot mL^{-1}$).

Statistical analysis

All data were recorded as mean ± standard deviation and analysed using the SPSS package program version 23.0 (SPSS Inc. Chicago, USA). One-way analysis of variance (ANOVA) and Duncan comparisons were performed to test for significant differences between raw and cooked vegetables.

RESULTS AND DISCUSSION

Total phenolic content. The study investigated the effect of different cooking methods and durations applied to chard, a plant frequently used in Turkish cuisine, on antioxidant activity. Phenolic compounds are important plant components with redox properties responsible for antioxidant activity. Hydroxyl groups in plant extracts are responsible for cleaning free radicals (Gamba et al. 2021).

The total phenolic content was measured in each extract using the Folin-Ciocalteu reagent and expressed as GAE. Results ranged from 1 157.67 mg GAE·$(100\ g)^{-1}$ to 2 611.33 mg GAE·$(100\ g)^{-1}$ (Table 1). The applied cooking methods were determined to affect the chard's phenolic content.

It was determined that the total phenolic content of the samples, which were cooked for 3.5 min in the microwave, increased compared to the raw chard samples. Similarly, it was determined that stir-frying applied for 5 min significantly increased the total phenolic content ($P < 0.05$).

The total phenolic content of the raw chard samples was determined to be 1 552.0 mg GAE·$(100\ g)^{-1}$ in this study. In a survey conducted with *Brassica* species in Spain, the total phenolic content was between 116.9 mg·$(100\ g)^{-1}$ and

Table 1. Total phenolic content and antioxidant activity in chard with different cooking methods compared to raw samples

Samples	Cooking time (min)	Total phenolic content [mg GAE·$(100\ g)^{-1}$]	Raw content (%)	FRAP [μmol Fe·$(100\ g)^{-1}$]	Raw content (%)
Raw	–	1 552.00 ± 299.82^{a}	100.00	4 120.33 ± 184.02^{c}	100.00
Boiling	5.0	1 186.67 ± 200.05^{a}	76.46	3 097.00 ± 127.57^{b}	75.16
	8.0	1 544.67 ± 211.01^{a}	99.53	4 203.33 ± 229.64^{c}	102.01
Microwave	3.5	2 611.33 ± 311.76^{b}	168.26	5 359.00 ± 236.26^{e}	130.06
	6.5	1 496.33 ± 107.93^{a}	96.41	4 623.67 ± 153.38^{d}	112.22
Stir-frying	5.0	2 434.33 ± 197.75^{b}	156.85	6 064.67 ± 252.87^{f}	147.19
	10.0	1 157.67 ± 33.83^{a}	74.59	2 656.33 ± 119.44^{a}	64.47

$^{a-f}$ different letters within columns indicate statistically significant differences ($P < 0.05$); total phenolic content and antioxidant activity shown as the mean ± standard deviation (SD); FRAP – ferric reducing antioxidant power; GAE – gallic acid equivalents

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158.8 mg·(100 g)$^{-1}$ (Lafarga et al. 2018). The 10-fold difference between these plants with similar structures may be due to growing conditions, geographical differences and differences in harvest time.

Francisco et al. (2010) stated that applying the boiling method significantly reduced the total phenolic content in their study with *Brassica* vegetables. In this study, no significant change was found in the total phenolic components of the boiled chard, regardless of the boiling time. In a study conducted with broccoli, it was determined that 28.1% and 28.4% of the phenolic component content were preserved in the microwave (5 min) and boiling (5 min) methods, respectively (Zhang and Hamauzu 2004). This study found that when boiling for 5 min and 8 min, the total phenolic content was 76.46% and 99.53%, respectively, and almost all phenolic components were preserved. In addition, the total phenolic compounds of chard cooked in the microwave for 3.5 min and 6.5 min were 168.26% and 96.41%, respectively. In other words, it was determined that the short microwave cooking time increased the amount of phenolic compounds (P < 0.05). The cell wall structure may be distorted due to the effect of electromagnetic radiation during microwave cooking. It is thought that this situation may cause an increase in phenolic compounds. However, phenolic compounds' structure may deteriorate as the cooking time increases.

The study conducted by Hossain et al. (2017) in Bangladesh with three different types of spinach determined that the total phenolic substance in the stir-fried spinach for 5 min was much higher than that boiled for 5 min. Similarly, in this study, it was determined that the chard sample [2 434.33 mg GAE·(100 g)$^{-1}$], which was stir-fried for 5 min, had much higher total phenolic substances than that boiled for 5 min [1 186.67 mg GAE·(100 g)$^{-1}$]. This may be because phenolic compounds are released due to the effect of heat during stir-frying. It is thought that during boiling, the phenolic compounds in the plant pass into the water and decrease.

A study conducted with cabbage samples taken from Trabzon in Türkiye examined the cooking methods' effects on the total phenolic content. It was concluded that the best cooking method in the total phenolic content was the microwave method, and the method with the lowest total phenolic content was the boiling method (Akdaş and Bakkalbaşı 2017). Due to the different methods and times used in this study, it was found that the amount of phenolic substances was higher in the chard samples that were stir-fried for 10 min and microwaved for 6.5 min. A study on chard determined that the total phenolic content in 8 min of boiling and 6.5 min of microwave cooking was 76% and 91%, respectively (Burri et al. 2017). In this study using the same durations, the total phenolic substances were found to be 99.53% in 8 min of boiling and 96.41% in 6.5 min of microwave. The change in the amount of phenolic substance depends on the presence of sugar, carotenoid and ascorbic acid in the plant and varies according to geographical variation (Burri et al. 2017).

Total antioxidant activity. Various methods have been developed to estimate the total antioxidant activity in vegetables. This study used two different methods (DPPH and FRAP) (Table 1, Figure 2). The principle of the FRAP method, which is one of these methods, is based on the high antioxidant activity of the plant extract and the conversion of Fe^{+3} ions to Fe^{+2} ions (Benzie and Szeto 1999).

As a result of the cooking methods applied to wild chard in this study, it was determined that the FRAP values of the samples were between 2 656.33 μmol·(100 g)$^{-1}$ and 6 064.67 μmol·(100 g)$^{-1}$ (Table 1). A survey conducted with cabbage showed that FRAP values were higher in microwave cooking than in boiling (Armesto et al. 2019). This result is similar to our study. In a survey conducted with plants native to the diet of India, it was found that boiling and microwave cooking decreased the FRAP value (Saikia and Mahanta 2013). In this study, 5 min of boiling method decreased the FRAP value, while 8 min did not change the FRAP value (P > 0.05). It was determined that the FRAP values of the chard samples increased in microwave cooking (P < 0.05). It is suggested that the difference in the cooked samples may affect the antioxidant activity.

The DPPH method is a colour change test that measures natural extracts' free radical scavenging potential. This method is widely used to measure the antioxidant activity of a substance due to its excellent reproducibility under certain conditions (Armesto et al. 2019). The free radical scavenging activity for the DPPH radical is expressed as IC_{50} in the extracts. All extracts have an inverse relationship between IC_{50} and antioxidant activity. The lower the IC_{50} value is, the higher the antioxidant potential of the extracts is (Senarathne et al. 2017).

Chang and Hsu (2000) reported that the DPPH values of potato leaves, which were boiled for 2 min at 100 °C, were close to that of the raw sample. This study found that the radical scavenging effect of the cooking method applied for 5 min and 8 min was significantly higher (P < 0.05). However, another study reported that the radical scavenging effect of broccoli flower samples decreased due to boiling and microwaving (Zhang and Hamauzu 2004). In this study, on the contrary, it was

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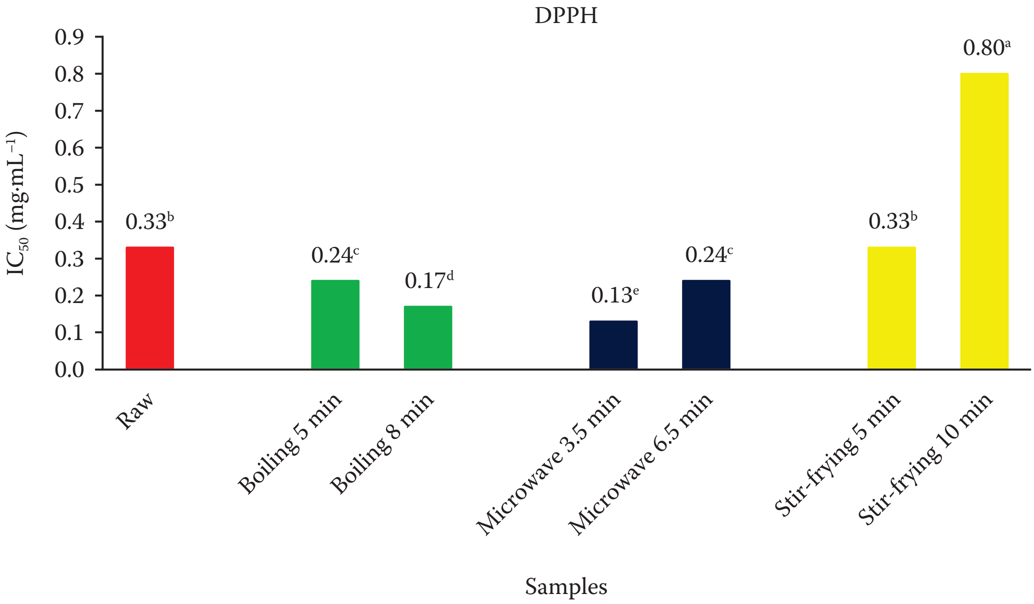

Figure 2. Effect of different cooking methods on DPPH radical scavenging activity of wild chard

[a–e] statistically significant differences (P < 0.05); antioxidant activity shown as the mean; DPPH – 2-diphenyl-1-picrylhydrazyl; IC_{50} – half maximal inhibitory concentration

determined that the radical scavenging effect of boiled and microwaved chard samples increased (P < 0.05).

A study conducted with cabbage determined that the antioxidant activity of microwave cooking was higher due to the DPPH method than boiling and stir-frying (Akdaş and Bakkalbaşı 2017). DPPH radical scavenging activity in this study varies according to the stir-frying time. As the time increases, the cleaning activity decreases. In addition, it was determined that DPPH radical scavenging activity increased in boiling and microwave-cooking methods (P < 0.05).

It is thought that the increase in the antioxidant activity of vegetables may be possible with four different factors: *i*) with the deterioration of the cell wall and cell structure, antioxidants are released and become active; *ii*) an increase in the number of antioxidants as a result of an increase in the reactions with the effect of heat; *iii*) losing the activity of enzymes involved in oxidation reactions, increasing the activity of antioxidants with the effect of heat; *iv*) as a result of the reactions, the antioxidant activity increases with the release of new antioxidants such as Maillard reaction products (Jiménez-Monreal et al. 2009).

However, it still needs to be determined to what extent each possible factor contributes to the increase in activity. Sultana et al. (2008) say that the vitamin losses associated with cooking vegetables can be compensated by the antioxidants that occur from cooking. When all these conditions are considered, it can be said that many factors affect the antioxidant activity. In particular, geographical structure, plant species and plant growing conditions effect antioxidant activity.

Correlation between total phenolic content and antioxidant activity. The correlation between total phenol content, DPPH (IC_{50}) and FRAP is given in Table 2. The total phenolic content significantly correlated to DPPH (IC_{50}) and FRAP with Pearson's correlation coefficients of –0.532 and 0.754, respectively. Many studies have found a correlation between the total phenolic content in the extracts of various vegetables

Table 2. Correlations between total phenolic content, FRAP, and DPPH

Analytical methods	Total phenolic content	FRAP	DPPH (IC_{50})
Total phenolic content	1	0.754**	–0.532*
FRAP	–	1	–0.588*
DPPH (IC_{50})	–	–	1

* P < 0.05, ** P < 0.01 FRAP; DPPH – 2-diphenyl-1-picrylhydrazyl; IC_{50} – half maximal inhibitory concentration; FRAP – ferric reducing antioxidant power

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and their antioxidant activity (Dini et al. 2013; Ramirez-Anaya et al. 2015). Our study supports other studies. This shows that phenolic contents are important antioxidants. Phenolic contents change antioxidant activity because they show antioxidant properties. Therefore, the total phenolic content change also changes the antioxidant activity.

CONCLUSION

As a result, wild chard's entire phenolic content and antioxidant activity are high. Total phenolic content and antioxidant activity may change with cooking. It was concluded that the best cooking method and time among the cooking methods applied to wild chard is 3.5 min microwave cooking. As a result of this study, we think that the preferability of the microwave as a cooking method can be increased. Considering the importance of the effects of antioxidants on health, it is necessary to pay attention to cooking methods. Regarding antioxidant activity, its content can be preserved or increased by determining plant-specific cooking methods and times. This is valuable in preventing or protecting from diseases.

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