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# INFLUENCE OF DIFFERENT COOKING METHODS ON BIOACTIVE PROPERTIES OF BROCCOLI

Research Article

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#### ABSTRACT

It is well known that heat treatments have negative effect on food components, especially vitamins and phenolic compounds. However, degradation of these compounds can be prevented with avoiding conventional techniques such as boiling. Broccoli (*Brassica oleracea Italica*) has been widely consumed horticultural plant which has high antioxidant potential because of being rich in terms of vitamin C content and phenolic compounds. In the current study, three cooking methods were used: boiling, steaming and sous vide cooking, the lowest loss of vitamin C content and TPC was determined in steamed broccoli. When compared with boiling, the sous vide technique caused less reduction in vitamin C content and TPC, however the loss was more than that of steaming (p<0,05). Moreover, the highest antioxidant activity was found in steamed broccoli. Steaming can be suggested as a more suitable method for cooking broccoli. On the other hand, total chlorophyll content increased with the heat treatment in comparison to fresh broccoli. The highest chlorophyll content was observed in 10 min steamed broccoli.

Keywords: Antioxidant Activity, Broccoli, Cooking Techniques, Total Phenolic Component, Vitamin C

JEL Classification Codes: L66, Z31

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## **INTRODUCTION**

Broccoli (Brassica oleracea Italica), belonging to Brassicaceae (or Cruciferae) family, is a horticultural product which has high nutritional value and bioactive compounds. Brassica vegetables such as broccoli have been related with reducing inflammation related diseases, and chronic gastritis risk (Wagner et al., 2013). Its health promoting properties such as anticancer and antioxidant activities result from high phenolic, carotenoids, vitamins (especially vitamin C) and glucosinolates content (Podsedek, 2007; Latté et al., 2011). Additionally, broccoli has been stated to induce the detoxification enzymes in mammals (Dominguez-Perles et al., 2011).

Antioxidant activity in broccoli is mainly associated with flavonoids and vitamins (Roy et al., 2009). Phenolic compounds have ideal chemical structure for free radical-scavenging activities and may have neuroprotective, cardio protective, antidiabetic, antiaging, anticancer effects (Khurana et al., 2013; Tomás-Barberán et al., 2016). Ascorbic acid, a water-soluble vitamin known as vitamin C, has also scavenging free radical activity and is a chelating and reducing agent (Bendich et al., 1986). Recommended nutrient intakes (RNIs) for vitamin C has been notified as 45 mg/day for an adult man (FAO, 2019). Broccoli is one of the richest vegetables in terms of vitamin C. Daily consumption of approximately 100 g of broccoli would meet the requirements for vitamin C, as recommended by FAO/WHO.

Broccoli can be consumed as fresh or cooked in salads or garnitures as well as a meal independently. Several methods such as boiling, steaming, microwave heating, baking, frying and sous vide can be used for cooking broccoli. Although the most common methods are generally boiling and steaming, using sous vide has become popular recently. Cooking methods affect the content and bioavailability of bioactive compounds such as phenolic compounds, chlorophyll, vitamins and carotenoids in vegetables (Puupponen-Pimia et al., 2003; Vallejo et.al., 2002). According to several studies, heat treatments generally result in decreasing bioactivity of foods because of healthpromoting compounds losses such as vitamin C and phenolic compounds (Lafarga et al., 2018; Lafarga et al., 2018; Martínez-Hernández et al., 2013b).

In this study, effects of three cooking methods (boiling, steaming, sous vide) on total phenolic, chlorophyll and vitamin C content and antioxidant activity of broccoli were investigated. Additionally, the relationship between antioxidant activity and phenolic content-vitamin C was evaluated.

#### METHODOLOGY

#### Method and Sample

Broccoli was purchased from a local market in Denizli-Turkey. All chemical and reagents were analytical grade and purchased from Sigma-Aldrich.

#### Sample Preparation

Boiling, steaming and sous-vide techniques were used for cooking samples. Temperatures and cooking time were predetermined by consumer perceptions. According to pretesting, boiling process was carried out for 5, 10 and 15 min at  $100^{\circ}$ C in a stainless steel pan with 1/10 (w/v) sample/water ratio, steaming process was performed for 5, 10 and 15 min in a steamer with the stainless steel steamer basket placed on the pot boiling water (one inch), make sure that the water did not touch the sample. Before sous vide cooking, samples were placed in vacuum bags and vacuumed. Then sous vide process carried out with sous vide device (Polyscience, Sous Vide Professional Chef Series, USA) at 90°C for 5, 10 and 15 min. After cooking, all samples were rapidly cooled and stored at  $+4^{\circ}$ C for further analysis.

#### Vitamin C Analysis

Vitamin C analysis was performed according to Dhakal et al. (2018) with some modifications. 5 g of each samples was weighted and homogenized with distilled water with ratio 1:9 (w:v) using a laboratory type blender. Following centrifugation (at 4500 rpm for 10 min, Nüve NF800R), the supernatants were collected. Before the injection into the HPLC, the supernatants were filtrated using a 0.45µm filter.

A micro syringe was used for injection of 20  $\mu$ l of last filtrate into the HPLC column. Mobil phase consisted of 0,02 N H<sub>2</sub>SO<sub>4</sub> which is HPLC purity. A HPLC device (SHIMADZU), column oven at 25°C (SHIMADZU CTO-20A), column Coregel 87H3 (7.8x300 mm), pump (SHIMADZU LC-20AD), degaser (SHIMADZU DGU-20A3), photo diode array (PDA) detector (SPD-M20A) at 254 nm were used for analysis. The mobile phase was isocratic with 1ml/min flow rate.

#### **Total Phenolic Content Analysis**

Methanolic extraction was used for total phenolic content and antioxidant activity analysis. 5 gr of each sample was mixed 45 ml of %90 methanol. Samples were homogenized using a laboratory type blender. The mixtures were centrifuged at 4500 rpm for 10 min and the supernatants were collected, then filtrated with filter paper. Filtrate was stored in a dark place until analysis.

Total phenolic content analysis was performed according to Singleton and Rossi (1965) with slight modification. 300  $\mu$ l of extract and 1500  $\mu$ l of Folinciocalteu solution (% 10 v/v) were mixed. 3 minute later 1200  $\mu$ l of Na<sub>2</sub>CO<sub>3</sub> was added and the mixture was incubated at room temperature in the dark for 2 hours. After incubation, the absorbance of samples was measured at 760 nm by using spectrophometer (T80, PG Ins. UK.). Each sample were analysed in triplicate and total phenolic compound were given as ppm gallic acid equivalent (ppm GAE).

#### **Antioxidant Analysis**

The antioxidant capacity analysis performed as described by Thaipong et al. (2006) with slight modification. 150  $\mu$ L of extracts and 2850  $\mu$ L of DPPH methanolic solution (whose absorbance is 1,1 at 515 nm) were mixed and the mixture were incubated at room temperature in the dark for 60 minutes. After incubation, the absorbance of samples was measured at 515 nm (Thermo Scientific, Genesys 10S UV-Vis). Each sample were analysed in triplicate and antioxidant activity were given as mmol trolox equivalent (mmol TE).

### **Chlorophyll Analysis**

The analysis of pigments was carried out according to the method suggested by Nagata and Yamashita (1992). 1 g of each samples was weighted and separately homogenized with 10 ml of a hexaneacetone mixture (6:4) until colour disappearing in samples. After centrifugation (at 6000 rpm for 10 min at +4°C), the absorbance of each supernatant was separately measured at 645 and 663 nm. Chlorophyll contents of samples were calculated using Eq. (1) and Eq. (2) as below:

$$\begin{aligned} Chlorophyll \ a \ (mg/100g) &= 0.999 A_{663} - 0.0989 A_{645} \end{aligned} \tag{1} \\ Chlorophyll \ b \ (mg/100g) &= -0.328 A_{663} + 1.77 A_{645} \end{aligned} \tag{2}$$

### **Statistical Analysis**

Data were analysed by SPPS software (ver. 23 SPSS Inc., Chicago, IL, USA). ANOVA and Tukey's mean comparison test at a significance level of 5% were used for statistical analysis.

### **RESULTS AND DISCUSSION**

Vitamin C content, total phenolic content and antioxidant activity results were given in Table 1. As seen from the Table 1, for all cooking methods vitamin C content, total phenolic content and antioxidant activity of broccoli were decreased with time.

Vitamin C content varied with cooking method and decreased with the increment of cooking time for all methods. The highest content of vitamin C was observed in fresh broccoli (p<0,05). Total loss percentages for vitamin C content, total phenolic content, and antioxidant activity of broccoli with cooking methods and time were shown in Table 2.

Among the cooking methods, the lowest loss of vitamin C was obtained from steamed broccoli, while the highest loss was in boiled broccoli in the current study. Vitamin C is a water soluble, light and temperature sensitive component. Therefore, vitamin C can decrease due to its character during the heat treatment (Gamboa-Santos et al., 2013). When compared with literature, many researchers have indicated that sous vide processing is the most suitable cooking method for reducing vitamin C losses, because oxidative degradation of vitamin C can be reduced by using sous vide technique (Lafarga et al., 2018; Baardseth et al., 2010; Lafarga et al., 2018; Martínez-Hernández et al., 2013b). In the current study, it was observed that steaming was more effective cooking method for reducing vitamin C losses. It can be explained that vacuuming may not be adequate for preventing oxidative degradation.

Method	Time (min)	Vitamin C Content (mg/kg)	Total Phenolic Content (mg GAE/kg)	Antioxidant Activity (mmol TE/g)
Raw	0	$637,\!61 \pm 10,\!66^{a}$	$2282,\!97 \pm 14,\!09^{a}$	$0,189 \pm 0,0028^{a}$
<b>S</b>	5	$514,46 \pm 6,49^{b}$	$2098,\!96 \pm 13,\!35^{\text{b}}$	$0,\!158\pm0,\!0026^{\text{b}}$
Sous	10	$406,03 \pm 2,95^{\circ}$	$1974, 17 \pm 6, 11^{\circ}$	$0,138 \pm 0,0008^{\circ}$
Vide	15	$289{,}51\pm4{,}59^{d}$	$1845,\!88\pm7,\!10^{\rm d}$	$0,\!119\pm0,\!0020^{d}$
	5	$600,73 \pm 2,37^{e}$	$2188,3\pm9,41^{\text{e}}$	$0,\!174\pm0,\!0006^{e}$
Steaming	10	$566{,}14\pm 4{,}45^{\rm f}$	$2085,\!37\pm8,\!38^{\rm f}$	$0{,}156 \pm 0{,}0015^{\rm f}$
	15	$515,99 \pm 5,02^{\mathrm{g}}$	$1981 \pm 9{,}98^{\text{g}}$	$0,\!144\pm0,\!0027^{g}$
	5	$363,\!12\pm 4,\!31^{h}$	$1692,\!47\pm9,\!10^{\rm h}$	$0{,}133\pm0{,}0010^{\rm h}$
Boiling	10	$208{,}56 \pm 17{,}67^{\rm i}$	$1284,\!82\pm24,\!02^{\rm i}$	$0,\!090\pm0,\!0047^{\rm i}$
	15	$150,\!14\pm 8,\!73^{j}$	$926{,}56 \pm 8{,}74^{j}$	$0,\!069\pm0,\!0023^{\rm j}$

# Table 1: Vitamin C Content, Total Phenolic Content, and Antioxidant Activity of Broccoli

Different letters in the same column indicate statistical difference (p <0.05).

Broccoli						
Method	Time	Vitamin C Loss	Total Phenolic Content	Antioxidant		
	(min)	(%)	Loss (%)	Activity		
				Loss (%)		
Raw	0	0	0	0		
	5	19,31	8,06	16,4		
Sous	10	26.22	12.52	26.00		
<b>T</b> 7' 1	10	36,32	13,53	26,98		
Vide	15	54,59	19,15	37,04		
	5	5,78	4,15	7,94		
Steaming	10	11,21	8,66	17,46		
	15	19,07	13,23	23,81		
	5	43,05	25,87	29,63		

Table 2: Loss Percentage of Vitamin C, Total Phenolic Content and Antioxidant Activity of
Broccoli

Boiling	10	67,29	43,72	52,38
	15	76,45	59,41	63,49

Maximum total phenolic content (TPC) was observed in fresh samples. Although all cooking techniques caused decrement in TPC, minimum loss was found in steamed, while maximum loss was observed in boiled samples. The loss of TPC in boiled samples could be explained that phenolics were largely leached into the cooking water (Zhang and Hamauzu 2004). TPC of samples decreased with increasing cooking time for all cooking techniques. Lafarga et al. (2018) have found that TPC decreased with both conventional and sous vide cooking method. On the other hand, Girgin and El (2015) have indicated that TPC increased with steaming, but decreased with boiling in cauliflower when compared to the fresh sample. Martinez-Hernandez et al. (2013a) have notified that all cooking methods except boiling increased TPC in broccoli. As similar with the results of the current study, Francisco et al. (2010) have stated that steaming and boiling reduced the TPC of Brassica vegetables. Additionally, many other researchers have obtained similar results with the current study (dos Reis et al., 2015a; Pellegrini et al., 2010; Lafarga et al., 2018). Decrement of TPC could be explained that heat treatments such as boiling, steaming or sous vide have degradation effect on TPC.

The highest antioxidant activity was obtained from fresh broccoli. Although all cooking methods negatively affected the antioxidant activity (p<0,05), steaming was found as the most suitable method for preserving the antioxidant activity. Lafarga et al. (2018) have stated that thermal processing reduced the antioxidant activity of some brassica vegetables. In their study, antioxidant activity of some brassica cultivars varied depending on cooking methods, while for some cultivars, cooking methods had no importance on reducing antioxidant activity. Dolinsky et al. (2016) have indicated that there were no differences between antioxidant activity of fresh and cooked broccoli. In a study, it was found that conventional cooking method decreased antioxidant activity, while sous vide cooking method increased antioxidant activity of broccoli (Kosewski et al., 2018). On the other

hand, increment in antioxidant capacity with different cooking methods such as boiling, steaming, microwave and sous vide was reported by many researchers (Turkmen et al., 2005; Lafarga et al., 2018; dos Reis et al., 2015b; Wachtel-Galor et al. 2008; Martínez-Hernández et al., 2013a). This increment may be explained with production of redox-active secondary plant metabolites or breakdown products and due to changing in plant cell wall structure and matrix modifications, release of antioxidants from intracellular proteins (Rechkemmer, 2007). In the current study, decreasing antioxidant activity may be related with thermal degradation of phenolic compound and vitamin C content. Additionally, leakage of antioxidants into the cooking water was the most important reason of this decreasing in boiling method which cause maximum antioxidant activity losses. On the other hand, losses of antioxidant activity were minimum in steaming method because there is not directly connect with water and temperature was lower than boiling and sous vide.

Chlorophyll is not only one of the important quality parameters for green vegetables, but also has health beneficial effects such as antimutagenic and anticarcinogenic activities (Nisha et al., 2004; Turkmen et al., 2006). Variation of total chlorophyll content of broccoli was shown in Figure 1. In this study, it was observed that the cooking time was also effective on the total chlorophyll content and the highest increase was observed in 10 minutes for the cooking methods used in the experiment. Yuan et al. (2009) have reported that different cooking methods such as boiling, stir-frying/boiling, stirfrying, and microwaving caused the loss of chlorophyll content, while steaming did not cause any significant loss of chlorophyll content. It was stated that microwave treatment was more effective to the total chlorophyll content in broccoli by dos Reis et al. (2015b), while boiling, steaming and sous vide treatments decreased. In another study dos Reis et al. (2015a) indicated that total chlorophyll content of broccoli was sorted from higher to lower as boiled, sous vide, microwave, steamed and fresh.

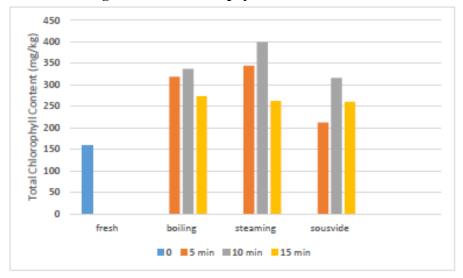


Figure 1: Total chlorophyll content of broccoli

#### CONCLUSION

Cooking techniques and time have important effects on bioactive compounds and antioxidant activity of broccoli. In this study, it was concluded that steaming of broccoli is the most appropriate method for reducing loss of vitamin C, phenolic and chlorophyll content and antioxidant activity. The lowest degradation of vitamin C and TPC was observed in steamed broccoli, while boiling caused the highest loss of vitamin C and TPC. Contrary to expectations, sous vide technique was not effective as steaming. Likewise, similar results were obtained for antioxidant activity. On the other hand, sous vide technique was found more suitable in comparison to boiling, however, not to steaming. In addition to these, total chlorophyll content is affected by the time of the cooking procedure. In the further studies, it will be useful to comparing the effects of cooking techniques especially sous vide with different parameters such as lower temperatures.

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### Ethical approval

This study is among the studies that do not require ethics committee approval due to not fall within the scope of research that requires one-to-one data collection from the participants and the data are obtained as secondary data.