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Investigation on the effects of cooking methods on anti-inflammatory and antioxidant activities of five mostly consumed vegetables in winter

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Abstract

The aim of this study is to investigate the effects of steaming and boiling on antioxidant and anti-inflammatory activity of five frequently consumed vegetables in winter. The vegetables were prepared by three different cooking methods including steaming, 5-minute boiling, 15-minute boiling to compare with their raw forms. Antioxidant capacity was measured with 1, 1-diphenyl–2 picrylhydrazyl (DPPH) radical scavenging method, anti-inflammatory activity was measured with 5-lipoxgenase inhibitory method, and the total phenolic content was detected after *in vitro* cooking process. The highest antioxidant activities among the raw vegetables were found in spinach (SR) (425.80 µg/mL, 12.83 mg/g) and broccoli (BR) (754.50 µg/mL, 7 mg/g). While boiling for 15 minutes and steaming slightly increased the antioxidant capacity in spinach and decreased it in broccoli; the highest activities among the mentioned cooking methods and vegetable samples were detected in these two vegetables. The lowest antioxidant activities were determined in leek (LR) (5662.0 µg/mL, 1.24 mg/g) and celery (CeR) (2796.0 µg/mL, 2.98 mg/g). In addition, the least affected vegetable from the cooking methods was spinach (S) (227.4-549.8 µg/mL). Cooking techniques have significative effects on the levels of phytochemical compounds and antioxidant capacities. It was observed that cooking methods increased the anti-inflammatory activity of the vegetables used in this study. Only broccoli (B) showed a decrease. The highest anti-inflammatory activity was found in the leek (L15) which was boiled for 15 minutes. Also, our results contribute to the databases that provide information about the effects of different cooking techniques on the antioxidant and anti-inflammatory potential of vegetables.

Keywords

Anti-inflammatory activity, Antioxidant activity, Cooking method, DPPH, Vegetable

Introduction

The concept of sustainable healthy life has gained importance with the changes in lifestyle in recent years. Consumption of healthy foods is the main component of a healthy lifestyle (Mori et al., 2009). Since growing evidence suggested the effect of nutrition on the healthy and long life, especially in recent years, the antioxidant intake has gained great importance in the developed countries (Finley et al., 2011). The consumption of fruits and vegetables significantly reduced the incidence of various chronic diseases including hypertension, cancer, stroke, and many others. Phytochemical compounds such as vitamins, flavonoids, and phenolic compounds are assumed to contribute to these health benefits (Grebmer et al., 2014; Grosso et al., 2013).

Green-leafy vegetables contain various types of active ingredients, which possess antioxidant activity. The majority of these active ingredients are vitamins and minerals, in addition to non-nutritive compounds such as quercetin. Overall, these active molecules play significant role in preventing diseases such as cancer (Wach et al., 2007; Abuajah et al., 2015).

Most vegetables are commonly cooked before being consumed in the food service industries, and homes. Cooking is known to cause some changes in the chemical composition through altering the bioavailability and concentration of phytochemical compounds such as vitamin C, carotenoids and polyphenols (Pellegrini et al., 2010).

The cooking process has been used since centuries to increase the flavor and extend the shelf life. However, high heat treatments often cause changes in physicochemical properties of the phytochemical compounds (Kosewski et al., 2018). Based on this, it is concluded that the phytochemical compositions of the vegetables change during heat treatment. Vegetables are usually consumed after being cooked in water or oil and at various temperatures or time periods. Therefore, cooking is a significant process that directly affects the final product obtained from vegetables (Soares et al., 2017; Miglio et al., 2008). Morales and Babel (2002) proposed four possible mechanisms to understand the reasons behind the change in the antioxidant activity of vegetables upon heat treatment: 1) release of higher amounts of antioxidant compounds due to the destruction of cell walls and cellular compartments, 2) formation of the new antioxidants or compounds 3) suppression of the antioxidants through thermal inactivation of oxidative enzymes, 4) production of the active radical scavengers.

Although the common sense suggests that the vegetables should be consumed unprocessed and preferably uncooked, there is growing evidence showing that the absorption of many nutrients is increased by cooking. While pharmacological effects of some vegetables are more prominent upon raw consumption, the others require appropriate cooking methods to boost the effect. However, further studies focusing on the effect of cooking on the nutritional content of vegetables remain needed (Link and Potter, 2004; Miglio et al., 2008). The effect of different cooking techniques on the organoleptic properties and phytochemical compound of vegetables have been investigated in several studies. Yet, different findings have been observed due to the processing conditions and the type of samples (Dolinsky et al., 2016; Iborra-Bernad et al., 2015; Palermo et al., 2014).

Vegetables contain a wide range of hydrophilic and lipophilic antioxidant compounds. Therefore, antioxidant activity should be evaluated by different methods since these compounds function synergistically and might stop free radicals in both phases (Abuajah et al., 2015).

Although there are various analytical methods developed to determine the antioxidant activity, using different methods and complicated systems, these methods are still in the development phase. While chemical principles are valid in these methods, an antioxidant shows high antioxidant activity with the selected measurement method, while the same antioxidant may show a lower activity with another method. Therefore, it is recommended to work with at least two methods to determine the antioxidant capacity and compare them (Aruoma, 2003).

Thus, the aim of this study is to establish a relationship among the type of cooking methods (raw, steamed, boiled), time of processing (raw, 5-minute boiling, 15-minute boiling, 5-minute steaming), the antioxidant capacity of five most consumed vegetables (broccoli, cabbage, celery, leek and spinach) in winter (Onur et al., 2017). It has been stated that the antioxidant activity is associated with the total phenolic content (Aruoma, 2003). Therefore, while selecting the method, TPC was performed in addition to DPPH measurement and it was aimed to determine whether the antioxidant activity was correlated with the anti-inflammatory (5-lipoxygenase inhibition) activity.

Materials and Methods

DPPH (1, 1-diphenyl–2 picrylhydrazyl) radical scavenging activity assay

DPPH radical scavenging activity was determined as described along with slight modifications (Brand-Williams et al., 1995). Shortly, the stock extracts were prepared in MeOH as 10 mg/mL concentrations and diluted to 5 mg/mL, 2.5 mg/mL, 1.25 mg/mL with MeOH. 10 µL of each dilution was mixed with 190 µL of 0.1 mM DPPH radical solution (Sigma-Aldrich) prepared in MeOH and each dilution was loaded into 96 well-plates, in triplicates. The plates were shaken gently and left in the room temperature and dark for 40 minutes until the measurements. Afterwards, absorbances were measured at 517 nm (Synergy HTX Biotech multimode ELISA reader). The half inhibitory concentration (IC_{50}) values, which show the concentration that caused a 50% inhibition of radical formation, were used to clarify the results. Gallic acid was used as the positive control. All tests were performed in triplicate.

Anti-inflammatory activity

5-lipoxygenase inhibition activity was performed according to the method suggested by Phosrithong and Nuchtavorn (Phosrithong and Nuchtavorn, 2016) with slight modifications described by Yıldırım et al. (Yıldırım et al., 2019). 10 µL of the extracts or standard indomethacin were added to 20 µL ethanol, 25 µL of sodium borate buffer solution (pH 9, 0.1 M), 20 µL pure water, and 25 µL of type V soybean lipoxygenase solution in the buffer (pH 9, 20.000 U/mL). The reaction mixture was pre-incubated for 5 minutes at 25 °C. Then, 100 µL of 0.6 mM linoleic acid solution was added to the solutions, mixed well and the change in absorbance at 234 nm was followed for 6 min. Each reaction was run for three times.

Determination of Total Phenolic Content (TPC)

Total phenolic contents in all extracts were established by Folin-Ciocalteu (FC) assay (Singleton and Rossi, 1965). Briefly, 5 µL from the test samples (10, 5, 2.5 and 1.25 mg/mL in MeOH) were mixed with 25 µL 0.2 N FC reagent and 145 µL distilled water the mixture were shaken quietly. Following six minutes incubation, 75 µL of Na_2CO_3 (7%) solution was added and shaken again. The plates were incubated at dark for two hours and at the room temperature. Absorbances at 765 nm were measured. Gallic acid was used as the standard. All tests were performed in triplicate.

Statistical Analysis

The data were given as means±standard deviations and statistically analyzed using one-way ANOVA followed by the Tukey's test. Multiple Comparison test with the confidence interval (CI) of 95% for each was performed using GraphPad Prism 5. Differences between means at $p<0.05$ levels were considered as statistically significant.

Results and Discussion

Many vegetables are consumed raw or cooked. Various cooking methods are applicable, but the most common ones for vegetables are steaming and boiling.

The antioxidant and the anti-inflammatory activity, also the total phenolic contents, results of the samples determined according to each cooking method were shown in Table 1.

Various evaluations were made according to these results. Vegetables with the highest antioxidant capacity were determined. The effect of the cooking method applied to each vegetable on antioxidant/anti-inflammatory activity and TPC were evaluated (Table 1).

The highest antioxidant activities and the total phenolic contents among raw vegetables were spinach (SR) (425.80 µg/mL, 12,83 mg/g) and broccoli (BR) (754.50 µg/mL, 7 mg/g). While boiling for 15 min and steaming slightly increased the antioxidant capacity in spinach, it decreased in broccoli. The highest activities among the mentioned cooking methods and raw vegetable samples were found in these two vegetables (Table 1).

The lowest antioxidant activities and the total phenolic contents among raw vegetables were determined in leek (LR) (5662.0 µg/mL, 1.24 mg/g) and celery (CeR) (2796.0 µg/mL, 2.98 mg/g). When cooking methods were compared, steaming the broccoli, cabbage and spinach; 15-minute boiling celery and leek were found to have the highest antioxidant activities. In addition, the vegetable that was the least affected by these cooking methods was spinach (S) (227.4-549.8 µg/mL) (Table 1).

It can be said that antioxidant activity is correlated with the total phenolic contents. It was also found that steaming increased TPC in broccoli (BV) and spinach (SV). Boiling process (5 minutes or 15 minutes) reduced TPC in broccoli (B), cabbage (C) and spinach (S). In other samples, TPC slightly increased or does not change with the boiling process (Table 1).

The highest antioxidant activity among all raw samples was observed in spinach (S). Similarly, the highest antioxidant activity among all cooked samples was detected in spinach (S) as well. When raw and boiled samples were compared, the antioxidant activity and TPC decreased in broccoli and cabbage upon boiling, while it increased in leek. When all samples were evaluated in terms of antioxidant activity, the vegetables with the highest antioxidant activity were found to be spinach (S) (227.4-549.8 µg/mL) and broccoli (B) (523.9-927.2 µg/mL) (Table 1).

There are other studies investigating the effect of steaming and boiling. Turkmen et al. (2005) studied the effect of cooking on the antioxidant activity of broccoli and found an increase after boiling, microwaving and steaming. In our study, the antioxidant effect of broccoli decreased by cooking. Paciulli et al. (2018) worked with brussels sprouts and zucchini to achieve more preferable physical (texture, color) and antioxidant results compared to steaming. Their results showed that there was a linear change in force/compression firmness for brussels sprouts and an increase in total phenolic content for zucchini when cooking time and/or temperature were changed. Steamed brussels sprouts showed significantly higher antioxidant activity values than air-steaming or raw. Miglio et al. (2008) investigated the effects of boiling and steaming on the phytochemical compounds and antioxidant activity of broccoli. The results showed an increased antioxidant activity upon steam cooking, possibly due to increased phytochemical compounds than the other cooking methods. An overall increase in antioxidant activity values were observed in all samples, possibly due to matrix softening and increased compounds that can be converted into new antioxidants. Also, in our results, DPPH values increased in boiling cabbage (C15) and broccoli (B15) for 15 minutes and decreased in celery (Ce15), leek (L15) and spinach (S15). In steaming, the DPPH values increased in broccoli (BV), spinach (SV), leek (LV) and celery (CeV), whereas decreased in cabbage (C). Similar to our results, Perez-Burillo et al. (2019) founded a relation among the type of processing, time of processing, antioxidant capacity, and the development of the Maillard reaction of 23 widely consumed vegetables. They determined that, by density, normal and well-cooked samples showed significantly higher antioxidant capacities than raw ones. In our samples, the activity increased in celery (Ce), leek (L) and spinach (S), yet decreased in cabbage (C) and broccoli (B) with the cooking process. The cooking process, the appliances and the tools used in our study have critical differences compared to the previous research. Additionally, the cooking time and other phytochemical componds found in the vegetables are known to influence the outcome. It is also seen that many other vegetables not included in our study are also affected by the cooking processes. Also, these differences in results can be explained by the change in the structure or concentration of phytochemical compounds with the cooking process.

In addition, conditions such as climate, growing and storage affect the chemical composition of the vegetable and thus its activity (Mazzeo et al., 2011).

Steaming or boiling can increase the antioxidant capacity of green leafy vegetables. In our results, the highest antioxidant activities of all raw vegetables were found in spinach (S) detected by DPPH. In addition, spinach was the least affected vegetable by the cooking processes. In a study, Hossein et al. also investigated the effect of different cooking methods on antioxidant capacity. They selected three spinach species and green leaved amaranth which are antioxidant rich. The results showed a significant increase in TPC, total flavonoid content (TFC) and reducing power (RP) in all samples. These results reveal the effects of different heat treatments on the antioxidant potential of vegetables. In raw, green leaf amaranth, water spinach leaf, and Indian spinach leaf had similar TPC, while garden spinach had the lowest. Both boiling and frying significantly increased the radical scavenging ability of DPPH compared to the raw ones (Hossain et al., 2017). As in their study, decreases were also found using the radical scavenging ability of DPPH by Martínez-Hernández et al. (2013) in boiled and steamed broccoli. Ramirez-Anaya et al. (2015) also determined the fat, moisture, TPC, eighteen phenolic compounds and antioxidant capacities of raw vegetables and compared them with the values obtained after cooking. Ultimately, antioxidant capacity measured by all cooking methods remained constant or increased, however TPC decreased. Compared with our findings, different results were obtained in the vegetables we studied, as seen in Table 1. While the antioxidant activity increased in some samples, the activity decreased in others after cooking.

Results from previous studies, which evaluated the effect of temperature on the TPC of vegetables are contradictory. Adefegha and Oboh (2011) found that steaming caused a significant increase in TPC and TFC of eight tropical leafy greens upon cooking. This is thought to be related to the breakdown of cell walls and the release of bioactive compounds. Conversely, Lafarga et al. (2018) investigated the antioxidant potential of some Brassica vegetables and the effect of steaming and sous-vide treatments on TPC. In most of the evaluated samples, no difference was found between phenolic compounds loss after steaming and sous-vide. Dolinsky et al. (2016) evaluated the effects of different cooking techniques on the polyphenols and antioxidants of vegetables. Zucchini, collard greens, cabbage, green beans, broccoli, carrots and tomatoes were subjected to different cooking techniques and compared with their raw forms. The results showed that the heat treatment had a significant effect on the antioxidant capacity and soluble polyphenolic content. They recommended steaming as the best cooking method for these vegetables to increase the level of both antioxidants and polyphenols. In accordance with these, in our results, steaming increased TPC in spinach (S) and broccoli (B), in which the greatest increase was observed in spinach (from 12.83 mg/g to 20.47 mg/g). At the same time, the increase in TPC is higher in the 15-minute boiling method compared to 5-minute boiling except leek (L).

It was observed that cooking methods increased the anti-inflammatory activity of the vegetables used in this study. Only broccoli showed a decrease. The highest anti-inflammatory activity was found in the leek (L15) which was boiled for 15 minutes. However, cabbage (C15), celery (Ce15) and spinach (S15) boiled for 15 minutes showed strong activity (respectively 0.05 mg/mL, 0.06 mg/mL and 0.07 mg/mL)

It is seen that the five vegetables used in this study are affected differently by the cooking processes. (Table 1)

Table 1. The antioxidant activity, the anti-inflammatory activity and the total phenolic content results

Samples	Method	Raw (R)	Boiling 5 min. (5)	Boiling 15 min. (15)	Steaming (V)
		IC_{50}, µg/mL			
B	DPPH	754.5±1.56^{b}	927.2±2.33^{d}	794.7±3.18^{c}	523.9±0.42^{a}
	Anti-lipoxgenase	0.18±0.00^{a}	0.42±0.00^{b}	0.39±0.00^{b}	0.17±0.04^{a}
	TPC (mg/g)	7.0±0.04^{bc}	4.99±0.06^{a}	6.91±0.09^{b}	8.43±0.19^{c}
C	DPPH	1421±14.85^{a}	4218±2.83^{d}	2240±34.65^{c}	1694±9.19^{b}
	Anti-lipoxgenase	0.60±0.00^{c}	0.15±0.00^{a}	0.05±0.00^{a}	0.38±0.00^{b}
	TPC (mg/g)	3.29±0.03^{c}	1.08±0.03^{a}	2.05±0.06^{b}	2.07±0.01^{b}
Ce	DPPH	2796±9.90^{c}	1481±9.90^{ab}	1129±22.63^{a}	1805±6.36^{b}
	Anti-lipoxgenase	0.59±0.00^{d}	0.24±0.00^{b}	0.06±0.00^{a}	0.54±0.01^{c}
	TPC (mg/g)	2.98±0.04^{b}	1.96±0.00^{a}	3.48±0.04^{c}	2.93±0.23^{b}
L	DPPH	5662±37.48^{d}	2077±6.36^{b}	1469±18.38^{a}	3493±0.71^{c}
	Anti-lipoxgenase	0.65±0.00^{c}	0.30±0.00^{b}	0.04±0.00^{a}	0.47±0.01^{bc}
	TPC (mg/g)	1.24±0^{ab}	2.30±0.03^{c}	1.40±0.04^{b}	0.81±0.04^{a}
S	DPPH	425.80±0.99^{c}	549.80±6.72^{d}	314.50±2.83^{b}	227.40±2.69^{a}
	Anti-lipoxgenase	0.05±0.00^{a}	0.16±0.00^{b}	0.07±0.01^{a}	0.28±0.00^{c}
	TPC (mg/g)	12.83±0.04^{b}	7.66±0.06^{a}	13.58±0.06^{b}	20.47±0.04^{c}
Control	Gallic acid (DPPH)	2.46±0.01			
	Indomethacine	0.02±0.00			

*Abbreviations: B, C, Ce, L and S show methanol extract obtained from vegetables; raw (R), boiling 5 minutes (5), boiling 15 minutes (15) and steaming (V) fractions. **Each value in the table is represented as mean ± SD. Different letter superscripts in the same line indicate significant differences ($p<0.05$).

Conclusion

The vegetables are significant part of nutrition and they are consumed either raw or cooked. The evident suggests that the preferred cooking method alters the antioxidant activity, which has critical roles in preventing diseases. Therefore, the importance of the cooking method elevates. In our study, the antioxidant activity increased in Ce, L and S samples upon cooking, yet decreased in B and C samples. Additionally, boiling time was detected to affect the activity and TPC. For example, the antioxidant activity of sample L was found to increase when boiled for 15 minutes compared to 5-minute boiling. In contrast, boiling spinach for 15 minutes boiling decreased the antioxidant activity compared to other cooking methods. Altogether, it is concluded that each vegetable was affected differently from the cooking processes. Our findings have identified that antioxidant activity is closely related to total phenolic content. Boiling methods and less preferred steaming methods are effective in increasing the antioxidant capacity of vegetables. The total increase in TPC values observed in this study partially contradicts the claim that cooked vegetables have lower nutritional value than raw ones. Heat treatment has a decisive effect on the bioactive component and antioxidants of vegetables. Our results also contribute to a database that provides information about the effects of different cooking methods on the antioxidant capacity of vegetables. It is important to provide enlightening information to the consumers and people who are responsible for the preparation and cooking of foods, especially by investigating the effects of home cooking methods on nutritive value. Additionally, considering the complex chemical matrix of the vegetables, further studies are needed to investigate the effect of cooking methods of vegetables on health.

Preparation of samples

Broccoli (*Brassica oleracea* var. *botrytis*), celery (*Apium graveolens*), cabbage (*Brassica oleracea* var. *capitata*), leek (*Allium ampeloprasum*) and spinach (*Spinacia oleracea*) were taken from a local market. Broccoli (B), cabbage (C), celery (Ce), leek (L) and spinach (S) were washed. Then, the vegetables were dried, 100 grams of them were weighed and they were prepared based on three different cooking methods.

Raw: 100 g of samples were cut into small pieces and placed in containers to be processed (BR, CR, CeR, LR, SR).

Boiling (5 minutes-15 minutes): 100 g of samples were added to 200 ml of boiled tap water and left to boil for five minutes in a covered stainless-steel cooker. For the 15 minutes boiling group, the same procedures were repeated and applied for 15 minutes. Afterwards, samples were filtered and allowed to cool (B5, C5, Ce5, L5, S5 and B15, C15, Ce15, L15, S15).

Steaming: 100 g of samples were added to sieves, which were placed on top of pots with 150 ml of boiled water. Samples cooked in the steam of the water for five minutes were removed and allowed to cool (BV, CV, CeV, LV, SV).

Extraction procedure

All samples were cut into small pieces by a blender. Samples were left to macerate for 48 hours by shaking occasionally in 200 ml methanol in the dark. After 48 hours later, the extracts were filtered. Then methanol was evaporated using a rotary evaporator. The extracts were kept at +4 °C until the time of use.

Compliance with Ethical Standards

Conflict of interest

The authors declared that for this research article, they have no actual, potential or perceived 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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