(cc) BY

Effect of different drying and cooking treatments on phytochemicals and antioxidant activity in broccoli: an experimental in vitro study

Amna HAMEED^{1,2}, Naeem FATIMA³, Hassan IFTIKHAR⁴, Asim MEHMOOD⁵, Muhammad Rizwan TARIQ^{6*} ^(D), Shinawar Waseem ALI⁶, Sajid ALI⁷, Muhammad SHAFIQ⁷, Zulfiqar AHMAD⁸, Umair ALI⁸, Madiha GHAZANFAR¹, Madiha IFTIKHAR^{1,2}, Waseem SAFDAR⁹, Asif AHMAD¹, Zunaira BASHARAT⁶, Zujaja UMER⁶, Maham KHALID¹⁰

Abstract

The current study aimed to assess broccoli's phytochemical profile and determine its antioxidant activity. Fresh broccoli was procured from a local farm and divided into two major treatment groups (cooked and dried). There were five sub-treatments in the cooking group (conventional, microwave, boiling, blanching, and steaming) and three treatments in the drying group (sun drying, freeze drying, and hot air drying). Ascorbic acid content, total carotenoids, TPC, TFC, and antioxidant activity were evaluated for every sub-treatment. The results depicted that the antioxidant constituent of cooked broccoli was complementary to dried broccoli samples. The maximum amount of ascorbic acid content (10.80 mg/100 g), total carotenoids (3.976 mg/g), total phenolic contents (225.80 mg/g), and the total flavonoid contents (42.92 mg/g) were found in freeze-dried broccoli. Freeze-dried broccoli also showed the highest antioxidant activity for DPPH free radical scavenging assay (62.45%), hydrogen peroxide radical scavenging assay (53.67%), and ABTS radical scavenging assay (65.84%).

Keywords: broccoli; cooking; drying; phytochemical; antioxidant activity.

Practical Application: Fresh vegetables are loaded with various nutritional and bioactive compounds that are short-lived food commodities. Their shelf life can be enhanced by little processing, such as drying or cooking, but processing results in nutritional loss, a significant challenge for the food industry. Investigating the neighbouring effect of various processing techniques on bioactive compounds and associated antioxidant activity will help reduce nutrient loss and optimize product quality.

1 Introduction

Vegetables are considered the great natural gift of nature as these provide us with many essential nutrients required for health maintenance and are significantly associated with reducing various chronic diseases (Drabińska et al., 2018). Among these vegetables, broccoli (*Brassica oleracea*) holds a unique position on the list of highly nutritious vegetables and has been in use for a long time. The name "broccoli" is derived from an Italian word "broccolo", which denotes the flowering top of a cabbage (Li et al., 2019). Broccoli is native to the Asia Minors and the Mediterranean. It is an excellent season crop of the mustard family and is closely related to cauliflower (Jacobo-Velázquez & Cisneros-Zevallos, 2012). Desirable broccoli has consistent flower buds and an excellent blue-green to green colour, and its global production touches a value of 22.3 million tonnes. China and India are leading areas for its production (Shultz, 2013). The high moisture content of fresh broccoli is associated with chemical, enzymatic, and microbiological processes, all of which can impair product quality and shelf life.

Broccoli is considered a potential source of several nutrients. It is observed to be rich in minerals, vitamins, dietary fiber and phytochemicals (e.g., glucosinolates and phenolic compounds). In recent years, its bioactive compounds, such as glucosinolates and their breakdown products, have gained increasing attention (Gu et al., 2022). Broccoli is relatively high in coenzyme Q10 which is fat-soluble antioxidant and contribute mainly to the energy manufacturing in our bodies (Sarmiento et al., 2016). Broccoli also possess other health-promoting compounds such as antioxidant and anti-carcinogenic compounds (Wang et al., 2020; Mandelova & Totusek, 2006). These diverse antioxidants

Received 02 Oct., 2022

Accepted 21 Jan., 2023

Department of Food Technology, Institute of Food and Nutritional Sciences, PMAS-Arid Agriculture University, Rawalpindi, Pakistan

²Department of Diet & Nutritional Sciences, Ibadat International University, Islamabad, Pakistan

³ Allied Hospital Faisalabad, Faisalabad, Pakistan

⁴Amna Inayat Medical College, Sheikhupura, Pakistan

⁵Department of Biosciences, Comsats University Islamabad, Sahiwal Campus, Sahiwal, Pakistan

⁶Department of Food Sciences, University of the Punjab, Quid-i-Azam Campus, Lahore, Pakistan

⁷ Faculty of Agricultural Sciences, University of the Punjab, Quid-i-Azam Campus, Lahore, Pakistan

⁸ Department of Food Science and Technology, Islamia University, Bahawalpur, Pakistan

⁹Department of Biological Sciences, National University of Medical Sciences, Rawalpindi, Pakistan

¹⁰ Punjab University College of Pharmacy, Allama Iqbal Campus, Lahore, Pakistan

^{*}Corresponding author: rizwan.foodsciences@pu.edu.pk

from broccoli have been reported a lot of health benefits which are frequently allied to control type 2 diabetes in diabetic patients, protection of various cancerious cells through blocking DNA damage (Qazi et al., 2010), cardiovascular disease and Alzheimer's disease (Fabbri & Crosby, 2016).

Drying is a well-known industrial processing technique for preserving fresh commodities and effectively extending shelf life (Xu et al., 2017). However, food deterioration and nutrient loss are unavoidable throughout the drying process. Many drying techniques, for instance, sun drying, hot air drying, and freeze drying can be applied to process vegetables. According to Mrkic et al. (2006), drying is responsible for the breakdown of the nutritional components of broccoli. In contrast, drying temperature and airflow rate also substantially affected bioactive constituents and antioxidant activity.

Prior to the addition of ingredients like salt, milk, pounded groundnuts, oil, butter, tomatoes, onions, chillies, and bicarbonate of soda, broccoli is typically blanched and boiled. They may also be dried, primarily to extend shelf life due to their perishable nature (Uusiku et al., 2010). Cooking and drying vegetables like broccoli can cause complex chemical and physical changes, including changes to cell wall structures (Palermo et al., 2014), an increase in the extractability of phenolic constituents, degradation of phenolic compounds due to oxidation and the breaking of covalent bonds (Nayak et al., 2015)., polymerization (formation of higher molecular weight compounds), and transesterification (redistribution of phenolic compounds), relative strength of these changes will decide whether the end result is a decrease or increase in the concentration of phenolic compounds or antioxidant activity (Korus, 2014). The effects of cooking and drying on the phenolic compounds of a few African vegetables have been studied, however the outcomes achieved vary greatly depending on the type of vegetable utilised, the cooking settings, the type of extraction used, and the research techniques employed (Lim & Murtijaya, 2007).

Furthermore, Physical state was an essential factor determining nutritional component degradation in broccoli (Jin et al., 2014). However, no comprehensive studies have been done on the impact of drying on the phytochemical profile and antioxidant activity of broccoli. In order to obtain a high-quality product, it is critical to select an appropriate drying procedure. The study's objective was to evaluate the impact of different cooking and drying techniques on the presence of phytochemicals and antioxidant activity of processed broccoli.

2 Materials and methods

2.1 Procurement of raw materials

Fresh green broccoli (*Brassica oleracea*) was collected from a local farm and stored in glass jars with closed lids at a temperature of 4 ± 1 °C for approximately 3-7 days during food processing and preservation laboratory, PMS-Arid Agriculture University, Rawalpindi. All analytical grade chemicals and reagents were purchased from Sigma-Aldrich (Germany).

2.2 Sample preparation

Fresh broccoli was adequately washed with clean water, and surface water was dried using a paper towel. After drying,

inedible parts were removed, broccoli was diced into little florets (approximately 2 cm in height and 1.5 cm in diameter). Samples were packed and stored for further use with sample codes labelled on them with reference to the treatment planned to be performed on them. Separate experimentation for drying and cooking techniques were carried out to evaluate their processing affect on various parameters. It is divided into two phases. Phase one for drying and phase two for cooking treatments.

2.3 Processing of broccoli

Inactivation of enzymes

Thermal inactivation of the enzymes was performed for all broccoli samples in capillary tubes (1 mm/20 μ L). Samples were heated in a circulating water bath (model 20B, Julabo, PA) The temperature was kept around 80-100 °C for a time period of 3-5 min. Heated samples were cooled immediately in ice-water.

Drying of broccoli samples

Broccoli samples were dehydrated through sun drying (SD), freeze-drying (FD), and hot air oven drying (HD). Diced broccoli was placed in cleaned stainless steel dishes covered with a net under good sunlight in January for ten days at approximately 13-24 °C. The final moisture content of sun-dried broccoli samples was (1.49 \pm 0.07%). The samples were freeze-dried by applying liquid nitrogen in a freeze drier (CRYODOS-50, Telestar Cryodos, Spain) for 10 hours. The vacuum pressure and cold trap temperature in the drying chamber were set to 50 Pa and -40 °C, respectively, following the method of Xu et al. (2020). The final moisture content for freeze-dried samples was $(1.29 \pm 0.06\%)$. For HD, fresh samples (500 g) were spread on the tray in a single layer and dried in a laboratory-scale hot air oven (DOF-230E, Bievopeak, Japan) at (40 °C) temperature for 72 hours. Their final moisture content of hot air dried samples was $(1.31 \pm 0.05\%)$.

2.4 Cooking of broccoli samples

Broccoli samples were cooked using various treatments, the processing conditions are mentioned in Table 1.

2.5 Extract preparation

Processed broccoli samples (10 g) from every treatment were homogenized with 15 mL of 80% methanol. After homogenization, samples were filtered, and extracts were stored in the refrigerator at (4 ± 1 °C) for further analysis. For further analysis, the mother extract prepared 1 mg/mL dilutions in 80% methanol.

2.6 Phytochemicals analysis of processed broccoli

Determination of ascorbic acid contents (ACC)

Ascorbic acid (Vitamin C.) was analyzed by a titrimetric method as described by Contreras-Calderón et al. (2011) with few modifications. Prepared broccoli extract (1mL) was mixed with 100 mL of 4% oxalic acid solution.

Cooking Method	Procedure
Conventional cooking (CC)	The broccoli sample (10 g) was added to (200 mL) boiled water and then cooked for 2 minutes with a closed lid to prevent water loss.
Microwave cooking (MC)	Diced broccoli (100 g) was taken in a glass bowl with 6 mL of distilled water. The bowl was covered with a cooking bag with a few holes and cooked in the 1000W commercial microwave oven (1025F1A, Midea Equipment, China) for 1.5 minutes.
Steaming (ST)	The diced sample was placed in a steam cooker in a covered dish and steamed for 7-8 minutes above boiling temperature under atmospheric pressure.
Blanching (BL)	Blanching is accomplished by immersing vegetables in hot water for 1-2 minutes at 75-95 °C.
Boiling (BO)	Diced broccoli (100 g) was added to the 150 mL of water just to attain the boiling in a stainless pan and then cooked for 5 minutes

Table 1. Cooking Techniques Used For Preservation of Broccoli Samples.

Determination of total carotenoid contents (TCC)

Total carotenoid content was analyzed using Loizzo et al. (2013). Total extracts were solubilized with methanol in water (8:2) n-hexane. Absorbance was measured at 460 nm using a spectrophotometer (Tecan-Sunrise, Austria), and the final carotenoid concentration was calculated by comparing it with the standard B-carotene curve.

Determination of total phenolic contents (TPC)

Total phenolic content was estimated using Jaiswal & Abu-Ghannam (2013). The spectrophotometer was used to measure the absorbance at 765 nm and results were estimated using the gallic acid standard curve.

Determination of total flavonoid contents (TFC)

Total Flavonoid content was assessed using Jaiswal & Abu-Ghannam (2013) protocol with few modifications. The stock solution was prepared by mixing 0.01 g extract with 10 mL methanol, and absorbance was measured at 510 nm using a spectrophotometer. TFC was determined by using quercetin as standard.

2.7 Chromatographic assessment of flavonoids

HPLC chromatogram was used to identify flavonoids in broccoli, and the results were analyzed by matching the retention time of standard compounds.

Sample preparation

For extract preparation, 2.5 g processed broccoli sample was mixed with 10 mL of methanol and 3mL of 25% HCl. The filtrate was diluted with 10 mL of methanol, and the volume was raised to 100 mL by using methanol. The prepared extract sample was injected at a rate of 1.5 mL/min into HPLC for further analysis. Extracts for all treatments were prepared by using the same method.

HPLC determination of flavonoids

High-Performance Liquid Chromatography (HPLC) involves the specification of reverse phase column C18 (4.6 mm \times 15 mm), Ultraviolet-Visible (SPD-10A) detector, column oven (CTO-10AS), system controller (SCL-10A) and auto injection

pump having model (SIL-10AS) was used to analyze the sample. Compounds were detected at a wavelength of 225-228 nm. Solvent, A water to solvent B methanol 80:20 with 0.5% phosphoric acid were used as a mobile phase by dual pumping system gradient. The temperature of the column was 30 °C. TFCs were determined by their corresponding retention time and curve formation against a standard, whereas kaempferol and quercetin were used as standards (Villiers et al., 2016).

2.8 Determination of antioxidant activity

DPPH free radical scavenging activity

Antioxidant activity was determined by the method of Zhang & Hamauzu (2004) with few variations using the 2,2,-diphenyl-2-picryl-hydrazyl (DPPH). A methanolic solution of extract (0.5 mL) was combined with 2 mL, 0.1 mmol/ L methanolic solution of DPPH. The resulting mixture was kept in the dark for about 30 minutes at room temperature. The absorbance of DPPH was then measured using a spectrophotometer at 516 nm. Using the Equation 1, antioxidant activity was expressed as a percentage of DPPH decrease

$$AA(\%) = \frac{Control \, absorbance - Sample \, absorbance}{Control \, absorbance} \times 100 \tag{1}$$

Hydrogen peroxide scavenging activity

The prepared extracts' hydrogen peroxide radical scavenging activity was determined using the method described by Ruch et al. (1989). The absorbance was measured at 230 nm, while the Equation 2 was used to calculate the hydrogen peroxide scavenging percentage;

$$Hydrogen\ peroxide\ Scavenging\ percentage = \frac{Abs_{Blank} - Abs_{Sample}}{Abs_{Blank}} \times 100$$
(2)

ABTS radical scavenging activity

The disappearance of ABTS radical action was used to determine the ABTS free radical scavenging activity (Re et al., 1999). The stock solution was prepared by mixing ABTS (7 mmol/L) and potassium persulfate solution (2.4 mmol/L) and placed in the dark for 12 to 16 hours at normal room temperature. The solution was diluted with 1 mL of ABTS solution and 60% methanol to obtain an absorbance of 0.70.02. 1 mL of extract was mixed with 1 mL of ABTS solution, and the absorbance

was measured at 734 nm. Equation 3 was used to calculate the ABTS radical scavenging activity.

$$Percentage Inhibitition Activity = \frac{Abs_{Blank} - Abs_{Sample}}{Abs_{Blank}} \times 100$$
(3)

2.9 Statistical analysis

Data were expressed using descriptive statistics (means, standard deviation) for triplicates of samples taken from each experimental treatment. Analysis of variance (ANOVA) was used to differentiate between data sets, and significant differences were considered when the means of the compared sets fluctuated at (P> 0.05) by using statistical software (Statistix 8.1).

3 Results and discussion

The results regarding the ascorbic acid content of cooked and dried broccoli are presented in Tables 2 and 3, respectively. In contrast, data regarding the antioxidant activity of cooked and dried broccoli are elucidated in Tables 4 and 5, respectively. Cooking significantly (p<0.05) influenced the ascorbic acid content of broccoli. Mean values illustrated less significant differences between samples cooked by MC (5.350 mg/100 g), CC (5.200 mg/100 g), and BO (4.630 mg/100 g). However, significant differences were noticed (p<0.05) between ST (7.593 mg/100 g) and BL (3.983 mg/100 g) broccoli. The results in Table 2 depicted a significant (p<0.05) effect of cooking treatments on the TCC of broccoli. The maximum amount of TCC was observed in ST (3.640 mg/g), CC (3.163 mg/g), and MC (3.163 mg/g), whereas the minimum values of TCC were retained by BL (2.683 mg/g) and BO (2.960 mg/g) samples. The TCC for MC and CC varied non-significantly. Similarly, TCC for BO and BL varied in a non-significant manner. However, the TCC of ST samples fluctuated significantly (p<0.05) compared to BO and BL treatments.

The Results for TPC of cooked broccoli showed significant variation for ST (191.75 mg/g) as compared to BO (98.08 mg/g) and BL (93.12 mg/g) treatments. However, a non-significant variance was established between MC (180.03 mg/g) and CC (178.24 mg/g). Similarly, non-significant variation was observed for BO (98.08 mg/g) and BL (93.12 mg/g) treatments. The results for TFC of cooked broccoli are described in Table 2, showing a significant (p<0.05) effect of various cooking temperatures on TFC. Results indicated that higher TFC was evident in ST (17.33 mg/g) followed by BO (6.910 mg/g) and MC (5.917 mg/g) treated samples. At the same time, the lowest TFC was retained by CC (4.860 mg/g) and BL (3.82 mg/g) broccoli samples.

The results regarding the ascorbic acid content of dried broccoli samples are shown in Table 3, which indicates the significant influence of drying treatments on studied samples. The highest ascorbic acid content was present in FD (10.80 mg/100 g), followed by HD sample (6.967 mg/100 g). At the same time, the lowest amount was retained by SD (6.387 mg/100 g) samples. A significant variation was observed between FD and HD samples, while HD and SD samples varied non-significantly. TCC for all drying treatments has been displayed in Table 3, which illustrates a significant variation. Among drying treatments, FD (3.976 mg/g) samples showed the best results for TPC, followed by HD (3.306 mg/g) and SD sample (3.266 mg/g) depicted in Table 3. FD samples showed significant variance with HD and SD,

Table 2. Phytochemical analysis (dry weight basis) of cooked brocolli treatments.

Cooking Treatments					
Parameters	CC	МС	BO	BL	ST
AAC (mg/100 g)	$5.20\pm0.43^{\rm bc}$	$5.35\pm0.31^{\mathrm{b}}$	4.63 ± 0.32^{cd}	$3.98\pm0.43^{\rm d}$	$7.59\pm0.36^{\rm a}$
TCC (mg/g)	$3.16\pm0.24^{\rm b}$	$3.16\pm0.15^{\rm b}$	$2.96\pm0.13^{\rm bc}$	$2.68\pm0.23^{\circ}$	$3.640\pm0.32^{\rm a}$
TPC (mg/g)	$178.24 \pm 3.37^{\rm b}$	$180.03 \pm 4.25^{\text{b}}$	$98.1 \pm 5.11^{\circ}$	$93.1 \pm 4.31^{\circ}$	191.75 ± 3.46^{a}
TFC (mg/g)	$4.86\pm0.36^{\rm d}$	$5.91\pm0.18^{\circ}$	$6.91\pm0.16^{\rm b}$	$3.81 \pm 0.31^{\text{e}}$	17.33 ± 0.335^{a}

Means ± S.D carrying different letters are statistically significant.

Table 3. Phytochemical analysis (dry weight basis) of dried broccoli treatments.

	Drying Treatments				
Parameters	SD	HD	FD		
AAC (mg/100 g)	$6.38 \pm 0.59^{\rm b}$	$6.96\pm0.47^{\mathrm{b}}$	10.80 ± 0.67^{a}		
TCC (mg/g)	3.26 ± 0.28^{b}	$3.30\pm0.19^{\rm b}$	$3.97\pm0.23^{\rm a}$		
TPC (mg/g)	$104.83 \pm 4.60^{\circ}$	176.85 ± 3.31^{b}	225.80 ± 4.04^{a}		
TFC (mg/g)	$8.56 \pm 0.32^{\circ}$	16.03 ± 0.31^{b}	42.92 ± 0.18^{a}		

Means \pm S.D carrying different letters are statistically significant.

Table 4. Antioxidant activity of cooked broccoli.

	Cooking Treatments				
Parameters	CC	MC	BO	BL	ST
DPPH (%)	53.77 ± 1.49^{bc}	54.95 ± 1.26^{b}	51.53 ± 0.81^{cd}	$49.89\pm2.03^{\rm d}$	58.80a±2.30ª
H ₂ O ₂ (%)	$46.73 \pm 1.15^{\text{b}}$	44.80 ± 2.16^{bc}	$42.46 \pm 1.30^{\circ}$	$41.90 \pm 2.21^{\circ}$	50.09 ± 1.63^{a}
ABTS (%)	57.60 ± 2.12^{bc}	$55.68 \pm 1.35^{\rm cd}$	$59.81 \pm 1.21^{\text{b}}$	$53.46\pm2.02^{\rm d}$	$64.28\pm2.95^{\rm a}$

Means ± S.D carrying different letters are statistically significant. DPPH: 2,2-diphenylpicrylhydrazyl; ABTS: 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid).

Table 5. Antioxidant activity of dried broccoli treatments.

Drying Treatments				
Parameters	SD	HD	FD	
DPPH (%)	$55.98 \pm 1.10^{\circ}$	$58.36\pm0.56^{\rm b}$	$62.45\pm1.56^{\text{a}}$	
H ₂ O ₂ (%)	$49.14\pm2.10^{\rm b}$	51.57 ± 1.50^{ab}	53.67 ± 2.21^{a}	
ABTS (%)	61.56 ± 1.41^{b}	63.69 ± 0.85^{ab}	$65.84 \pm 1.54^{\rm a}$	

Means ± S.D carrying different letters are statistically significant.

whereas non-significant variation was observed in HD and SD treated samples. The highest value of TPC was showed observed in FD (225.80 mg/g), followed by HD (176.85 mg/g), and SD (104.83 mg/g) treatments. All treatments showed significant variation from each other. Among all drying treatments, the highest TFC was found in FD (42.92 mg/g), while the lowest TFC was in SD (8.567 mg/g) samples. Statistically significant (p<0.05).difference was observed among all drying treatments. Statistical analysis intended for DPPH free radical scavenging activity is illustrated in Table 4. The Highest antioxidant activity (58.80%) was presented by ST, followed by MC (54.95%), CC (54.95%), and BO (51.53%) samples.

The blanched sample showed the lowest DPPH radical scavenger (49.89%). The results relating to broccoli extracts' hydrogen-peroxide free radical inhibition activity exhibited a significant (p<0.05) variation, according to Table 4. SD samples showed excellent inhibition activity (50.09%) among all treatments. The inhibition activity for CC, MC, BO, and BL was 46.73%, 44.80%, 42.46%, and 41.90%, respectively.

Similarly, for the ABTS assay, ST broccoli showed the highest radical scavenging activity (64.28%), whereas the lowest activity was observed in BL (53.46%) samples. For BO, CC, and MC treatments, ABTS activity was 59.81%, 57.60%, and 55.68%, respectively. It is evident from Table 4 that ABTS activity varied non-significantly among MC and CC, while significant variance was observed for other treatments.

The results for the DPPH assay, H_2O_2 free radical inhibition, and ABTS assay of dried broccoli are presented in Table 5. The best results were shown by FD (62.45%), followed by HD (58.36%), and SD (55.98%) extracts. The maximum radical scavenging activity was found in FD (53.67%) and, afterwards, HD (51.57%) and SD (49.14%) samples. ABTS radical scavenging power for all tested samples of broccoli manifested that the best antioxidant activity was observed in low-temperature FD samples (65.84%). In contrast, HD and SD samples showed 63.69% and 61.56%, respectively.

4 Discussion

The Non-significant effect was observed for broccoli samples treated through FD, HD, and SD. Steamed samples retained the highest ascorbic acid content attributed to minor leaching and heat stability. It was observed that cooking with little water for a short duration, as in MC and ST, leads to vitamin C preservation. Roselló-Soto et al. (2015) state that ascorbic acid loss during CC, Bo, and BL is associated with water solubility and percolation. Furthermore, Ansorena et al. (2013) reported that ascorbic acid reduction is interlinked with the quantity of water utilized for cooking, heating temperature, and cooking interval. The result of our studies showed maximum amount of ascorbic acid was retained by FD samples. Similar results were achieved by Cai et al. (2016), who observed that FD retains various health components. During drying, loss of vitamin C is ascribed to thermal degradation, and HD and SD result in oxidation of ascorbic acid content (Gan et al., 2017).

Cooking showed little effect on the retention of β –carotene; however other carotenes may also be lost (Ismail et al., 2004). ST and MC exerted a non-significant effect on TCC, whereas the β -carotene and lutein levels increased, and violaxanthin levels declined. β -carotene was somewhat heat labile and vanished in the first sixty seconds of cooking. All could be done due to the alteration of *cis* isomers of lutein to trans form (Updike & Schwartz, 2003).

Moreover, reduction in TCC is ascribed to carotenoid loss or transformation to cis isomers. The transformed β-carotene is the main component in broccoli, but thermal processing affects it and converts all the trans-isomers to cis forms. In dried vegetables, chemical changes occur due to the direct heating and oxidation process. This isomerization can be induced via temperature, and exposure to light results in loss of antioxidant activity (Iribe-Salazar et al., 2015). ST and MC retained maximum TPC because of controlled heating conditions. Prolonged boiling is responsible for forming phenolic-proteins complexes leading to reduced antioxidant capacity. On the other hand, MC preserves bioactive components in the tissues of cooked vegetables (Sultana et al., 2008). The results of our analysis were in agreement with those of López-Berenguer et al. (2007), who stated that microwave heating does not stimulate the release of polyphenols. Furthermore, in comparison to boiling, microwave cooking is responsible for the higher antioxidant activity of cooked vegetables.

Drying is associated with high temperature that deactivates various enzymes and inhibits the loss of phenolic acids. Plant polyphenols simply undergo oxidation and change product attributes. In this process, a direct heat source is used to remove the product's moisture content and dry it, which would somewhat degrade phenolic compounds. On the other hand, low-temperature freeze drying gave maximum retention of total phenolic content because this procedure used a vacuum for evaporation of moisture from a sample, which caused low and minimal nutrition loss (Mazzeo et al., 2011). Processing treatments have a more significant influence on the TFC of processed broccoli samples. It was determined that antioxidants and flavonoids in broccoli florets and stems decreased due to bioactive component leaching. Furthermore, cooking intervals also affected antioxidants, regardless of the cooking method used (Roy et al., 2007). This thermal cooking disrupts the cell walls and cell membranes of vegetables. It causes the discharge of phytochemicals from the insoluble part of broccoli, which proliferates the pool of flavonoids and phenolics. At the same time, boiling and blanching cause the leaching of some flavonoids and gave acceptable results (Gliszczyńska-Świgło et al., 2006).

According to Scalzo et al. (2008), flavonoids loss is attributed to polymerization during high temperature treatments. It was observed that quercetin and kaempferol were normally preserved in FD because vacuum drying, which caused the minimum loss of bioactive compounds. HD and SD are liable for degradation or loss of flavonoids due to prolong disclosure of broccoli under uncontrolled temperature and time (Bernhardt & Schlich, 2006).

A non-significant variation in DPPH activity was observed among MC and CC; however, ST, BL, and BO treatments significantly affected broccoli's antioxidant activity. Broccoli contains a variety of beneficial compounds. A few of them are heat labile, while others are not. Due to indirect contact with the heating system, steamed samples presented the highest antioxidant activity. No leaching of phytochemicals ensued, which impart higher DPPH free radical scavenging activity. Various cooking methods may have diverse effects on different components. CC broccoli and MC samples showed less activity than ST due to the leaching effect (Borowski et al., 2016).

The results of our studies showed maximum DPPH radical scavenging activity for FD samples per the literature by Paciulli et al. (2018). The lowest antioxidant activity was noticed in SD broccoli due to uncontrolled processing conditions (Guillén et al., 2017). Hydrogen peroxide free radical scavenging activity for all cooking treatments showed significant effects except for BO and BL, that varied non-significantly. Except for some phenolic losses, cooking had no adverse effect on vegetables' total antioxidant activity or total phenolic content. The intensification in hydrogen peroxide radical scavengers could be ascribed to increased soluble flavonoids released from the cell matrix due to thermal processing (Murador et al., 2016).

On the contrary, carotenoids, which are lipid-soluble antioxidants, were found to be responsible for up to 20% of the overall antioxidant activity of Brassica plants. Steaming of broccoli led to a superficial increase in hydrogen peroxide activity because of the production of redox-active secondary metabolites of the plant. It is highly related to the liberation of naturally occurring antioxidants from intracellular matrix proteins, matrix modifications, plant cell wall changes and more proficient release of antioxidants through homogenization of sample (Zhong et al., 2015). There is a synergistic approach between the diverse structures of some vegetable peroxidases that sometimes boost pro-oxidant activity (Ozgen et al., 2006). FD broccoli samples provided the highest values for hydrogenperoxide radical scavenging activity due to the maximum retention of antioxidants at low temperatures (Paciulli et al., 2015). Conversely, FD is an expensive process because of liquid nitrogen utilization. This process can be replaced by high-temperature drying techniques of HD and SD that cause less retention of antioxidant activity than FD but are in an acceptable range. Aqua thermal processing reduced antioxidant activity as evaluated by the ability to quench the ABTS free radical, resulting in antioxidant vegetable losses. This could be an effect of various measurement procedures. The antioxidizing activity of broccoli decreased after BL and BO, due to the leaching of phytonutrients that altered antioxidant properties (Buratti et al., 2020). On the other hand, BO usually results in less polyphenol loss than BL, with green vegetables losing the most. Steamed tissues of vegetables are not in direct contact with water. This indirect aqua thermal cooking procedure minimizes the leaching of soluble antioxidants in water and limits thermal degradation. Therefore, it possessed maximum ABTS free radical scavenging activity than BO and

BL (Dolinsky et al., 2016). Broccoli should be ingested as often as possible with as little processing as feasible to get the benefits of its bioactive compounds (Amin et al., 2006).

Among all drying treatments applied to broccoli, FD samples illustrated the minor damage to ABTS scavenging activity due to low temperature that reserved maximum bioactive components in broccoli. ABTS radical scavenging activity was less in HD samples than FD due to the utilization of a controlled temperature of high intensity to dry produce. It is a low-cost drying procedure but significantly affects ABTS radical scavenging activity because of prolonged exposure to high temperatures (Mahn & Rubio, 2017).

5 Conclusion

Thermal processing dramatically affects the antioxidant properties of fresh commodities due to various polymerization and conversion reactions of these bioactive constituents. The current study showed that steaming retained more antioxidant components than other cooking treatments. This could be ascribed to controlled heating conditions, indirect heating systems and short cooking intervals. Cooking in water seems to cause leaching of antioxidants that amplifies with cooking time. Using cooking water for other purposes, such as a base for soups, gravies, and other applications, should be considered for the optimal intake of antioxidants from cooked vegetables.

References

- Amin, I., Norazaidah, Y., & Hainida, K. E. (2006). Antioxidant activity and phenolic content of raw and blanched *Amaranthus* species. *Food Chemistry*, 94(1), 47-52. http://dx.doi.org/10.1016/j. foodchem.2004.10.048.
- Ansorena, D., Barriuso, B., Cardenia, V., Astiasarán, I., Lercker, G., & Rodriguez-Estrada, M. T. (2013). Thermo-oxidation of cholesterol: effect of the unsaturation degree of the lipid matrix. *Food Chemistry*, 141(3), 2757-2764. http://dx.doi.org/10.1016/j.foodchem.2013.04.129. PMid:23871021.
- Bernhardt, S., & Schlich, E. (2006). Impact of different cooking methods on food quality: retention of lipophilic vitamins in fresh and frozen vegetables. *Journal of Food Engineering*, 77(2), 327-333. http://dx.doi. org/10.1016/j.jfoodeng.2005.06.040.
- Borowski, J., Narwojsz, J., Borowska, E. J., & Majewska, K. (2016). The effect of thermal processing on sensory properties texture attributes and pectic changes in broccoli. *Czech Journal of Food Sciences*, 33(3), 254-260. http://dx.doi.org/10.17221/207/2014-CJFS.
- Buratti, S., Cappa, C., Benedetti, S., & Giovanelli, G. (2020). Influence of cooking conditions on nutritional properties and sensory characteristics interpreted by e-senses: case-study on selected vegetables. *Foods*, 9(5), 607. http://dx.doi.org/10.3390/foods9050607. PMid:32397489.
- Cai, C., Miao, H., Qian, H., Yao, L., Wang, B., & Wang, Q. (2016). Effects of industrial pre-freezing processing and freezing handling on glucosinolates and antioxidant attributes in broccoli florets. *Food Chemistry*, 210, 451-456. http://dx.doi.org/10.1016/j. foodchem.2016.04.140. PMid:27211670.
- Contreras-Calderón, J., Calderón-Jaimes, L., Guerra-Hernández, E., & García-Villanova, B. (2011). Antioxidant capacity phenolic content and vitamin C in pulp peel and seed from 24 exotic fruits from Colombia. *Food Research International*, 44(7), 2047-2053. http:// dx.doi.org/10.1016/j.foodres.2010.11.003.

- Dolinsky, M., Agostinho, C., Ribeiro, D., Rocha, G. D. S., Barroso, S. G., Ferreira, D., Polinati, R., Ciarelli, G., & Fialho, E. (2016). Effect of different cooking methods on the polyphenol concentration and antioxidant capacity of selected vegetables. *Journal of Culinary Science & Technology*, 14(1), 1-12. http://dx.doi.org/10.1080/1542 8052.2015.1058203.
- Drabińska, N., Ciska, E., Szmatowicz, B., & Krupa-Kozak, U. (2018). Broccoli by-products improve the nutraceutical potential of glutenfree mini sponge cakes. *Food Chemistry*, 267, 170-177. http://dx.doi. org/10.1016/j.foodchem.2017.08.119. PMid:29934153.
- Fabbri, A. D., & Crosby, G. A. (2016). A review of the impact of preparation and cooking on the nutritional quality of vegetables and legumes. *International Journal of Gastronomy and Food Science*, 3, 2-11. http://dx.doi.org/10.1016/j.ijgfs.2015.11.001.
- Gan, S. H., Ng, M. X., Tham, T. C., Chua, L. S., Aziz, R., Baba, M. R., Abdullah, L. C., Ong, S. P., & Law, C. L. (2017). Drying characteristics of Orthosiphon stamineus Benth by solar-assisted heat pump drying. *Drying Technology*, 35(14), 1755-1764. http://dx.doi.org/10.1080/0 7373937.2016.1275673.
- Gliszczyńska-Świgło, A., Ciska, E., Pawlak-Lemańska, K., Chmielewski, J., Borkowski, T., & Tyrakowska, B. (2006). Changes in the content of health-promoting compounds and antioxidant activity of broccoli after domestic processing. *Food Additives and Contaminants*, 23(11), 1088-1098. http://dx.doi.org/10.1080/02652030600887594. PMid:17071511.
- Gu, H.-F., Mao, X.-Y., & Du, M. (2022). Metabolism, absorption, and anti-cancer effects of sulforaphane: An update. *Critical Reviews in Food Science and Nutrition*, 62(13), 3437-3452. http://dx.doi.org/1 0.1080/10408398.2020.1865871. PMid:33393366.
- Guillén, S., Mir-Bel, J., Oria, R., & Salvador, M. L. (2017). Influence of cooking conditions on organoleptic and health-related properties of artichokes green beans broccoli and carrots. *Food Chemistry*, 217, 209-216. http://dx.doi.org/10.1016/j.foodchem.2016.08.067. PMid:27664628.
- Iribe-Salazar, R., Caro-Corrales, J., Hernández-Calderón, O., Zazueta-Niebla, J., Gutiérrez-Dorado, R., Carrazco-Escalante, M., & Vázquez-López, Y. (2015). Heat transfer during blanching and hydrocooling of broccoli florets. *Journal of Food Science*, 80(12), E2774-E2781. http://dx.doi.org/10.1111/1750-3841.13109. PMid:26502365.
- Ismail, A., Marjan, Z. M., & Foong, C. W. (2004). Total antioxidant activity and phenolic content in selected vegetables. *Food Chemistry*, 87(4), 581-586. http://dx.doi.org/10.1016/j.foodchem.2004.01.010.
- Jacobo-Velázquez, D. A., & Cisneros-Zevallos, L. (2012). An alternative use of horticultural crops: stressed plants as biofactories of bioactive phenolic compounds. *Agriculture*, 2(3), 259-271. http://dx.doi. org/10.3390/agriculture2030259.
- Jaiswal, A. K., & Abu-Ghannam, N. (2013). Degradation kinetic modelling of color texture polyphenols and antioxidant capacity of York cabbage after microwave processing. *Food Research International*, 53(1), 125-133. http://dx.doi.org/10.1016/j.foodres.2013.04.007.
- Jin, X., Oliviero, T., van der Sman, R. G. M., Verkerk, R., Dekker, M., & Van Boxtel, A. J. B. (2014). Impact of different drying trajectories on degradation of nutritional compounds in broccoli (*Brassica oleracea* var italica). *Lebensmittel-Wissenschaft* + *Technologie*, 59(1), 189-195. http://dx.doi.org/10.1016/j.lwt.2014.05.031.
- Korus, A. (2014). Effect of preliminary processing, method of drying and storage temperature on the level of antioxidants in kale (*Brassica oleracea* L. var. *acephala*) leaves. *Lebensmittel-Wissenschaft* + *Technologie*, 44(8), 1711-1716. http://dx.doi.org/10.1016/j.lwt.2011.03.014.
- Li, Z., Mei, Y., Liu, Y., Fang, Z., Yang, L., Zhuang, M., Zhang, Y., & Lv, H. (2019). The evolution of genetic diversity of broccoli cultivars in

China since 1980. *Scientia Horticulturae*, 250, 69-80. http://dx.doi. org/10.1016/j.scienta.2019.02.034.

- Lim, Y. Y., & Murtijaya, J. (2007). Antioxidant properties of *Phyllanthus amarus* extracts as affected by different drying methods. *Lebensmittel-Wissenschaft* + *Technologie*, 40(9), 1664-1669. http://dx.doi. org/10.1016/j.lwt.2006.12.013.
- Loizzo, M. R., Pugliese, A., Bonesi, M., De Luca, D., O'Brien, N., Menichini, F., & Tundis, R. (2013). Influence of drying and cooking process on the phytochemical content antioxidant and hypoglycaemic properties of two bell *Capsicum annum* L. cultivars. *Food and Chemical Toxicology*, 53, 392-401. http://dx.doi.org/10.1016/j.fct.2012.12.011. PMid:23266504.
- López-Berenguer, C., Carvajal, M., Moreno, D. A., & García-Viguera, C. (2007). Effects of microwave cooking conditions on bioactive compounds present in broccoli inflorescences. *Journal of Agricultural and Food Chemistry*, 55(24), 10001-10007. http://dx.doi.org/10.1021/ jf071680t. PMid:17979232.
- Mahn, A., & Rubio, M. P. (2017). Evolution of total polyphenols content and antioxidant activity in broccoli florets during storage at different temperatures. *Journal of Food Quality*, 2017, 3742183. http://dx.doi. org/10.1155/2017/3742183.
- Mandelova, L., & Totusek, J. (2006). Chemoprotective effects of broccoli juice treated with high pressure. *Czech Journal of Food Sciences*, 24(1), 19-25. http://dx.doi.org/10.17221/3289-CJFS.
- Mazzeo, T., N'Dri, D., Chiavaro, E., Visconti, A., Fogliano, V., & Pellegrini, N. (2011). Effect of two cooking procedures on phytochemical compounds total antioxidant capacity and colour of selected frozen vegetables. *Food Chemistry*, 128(3), 627-633. http://dx.doi. org/10.1016/j.foodchem.2011.03.070.
- Mrkic, V., Cocci, E., Rosa, M. D., & Sacchetti, G. (2006). Effect of drying conditions on bioactive compounds and antioxidant activity of broccoli (*Brassica oleracea* L). *Journal of the Science of Food and Agriculture*, 86(10), 1559-1566. http://dx.doi.org/10.1002/jsfa.2554.
- Murador, D. C., Mercadante, A. Z., & de Rosso, V. V. (2016). Cooking techniques improve the levels of bioactive compounds and antioxidant activity in kale and red cabbage. *Food Chemistry*, 196, 1101-1107. http://dx.doi.org/10.1016/j.foodchem.2015.10.037. PMid:26593594.
- Nayak, B., Liu, R. H., & Tang, J. (2015). Effect of processing on phenolic antioxidants of fruits, vegetables, and grains: a review. *Critical Reviews in Food Science and Nutrition*, 55(7), 887-919. http://dx.doi.org/10. 1080/10408398.2011.654142. PMid:24915381.
- Ozgen, M., Reese, R. N., Tulio, A. Z. Jr., Scheerens, J. C., & Miller, A. R. (2006). Modified 2 2-azino-bis-3-ethylbenzothiazoline-6-sulfonic acid (ABTS) method to measure antioxidant capacity of selected small fruits and comparison to ferric reducing antioxidant power (FRAP) and 2 2 '-diphenyl-1-picrylhydrazyl (DPPH) methods. *Journal of Agricultural and Food Chemistry*, 54(4), 1151-1157. http://dx.doi.org/10.1021/jf051960d. PMid:16478230.
- Paciulli, M., Dall'Asta, C., Rinaldi, M., Pellegrini, N., Pugliese, A., & Chiavaro, E. (2018). Application and optimization of air-steam cooking on selected vegetables: impact on physical and antioxidant properties. *Journal of the Science of Food and Agriculture*, 98(6), 2267-2276. http://dx.doi.org/10.1002/jsfa.8715. PMid:28984926.
- Paciulli, M., Ganino, T., Pellegrini, N., Rinaldi, M., Zaupa, M., Fabbri, A., & Chiavaro, E. (2015). Impact of the industrial freezing process on selected vegetables—part I: structure texture and antioxidant capacity. *Food Research International*, 74, 329-337. http://dx.doi. org/10.1016/j.foodres.2014.04.019. PMid:28411999.
- Palermo, M., Pellegrini, N., & Fogliano, V. (2014). The effect of cooking on the phytochemical content of vegetables. *Journal of the Science of*

Food and Agriculture, 94(6), 1057-1070. http://dx.doi.org/10.1002/jsfa.6478. PMid:24227349.

- Qazi, A., Pal, J., Maitah, M., Fulciniti, M., Pelluru, D., Nanjappa, P., Lee, S., Batchu, R. B., Prasad, M., Bryant, C. S., Rajput, S., Gryaznov, S., Beer, D. G., Weaver, D. W., Munshi, N. C., Goyal, R. K., & Shammas, M. A. (2010). Anticancer activity of a broccoli derivative sulforaphane in barrett adenocarcinoma: potential use in chemoprevention and as adjuvant in chemotherapy. *Translational Oncology*, 3(6), 389-399. http://dx.doi.org/10.1593/tlo.10235. PMid:21151478.
- Re, R., Pellegrini, N., Proteggente, A., Pannala, A., Yang, M., & Rice-Evans, C. (1999). Antioxidant activity applying an improved ABTS radical cation decolorization assay. *Free Radical Biology & Medicine*, 26(9-10), 1231-1237. http://dx.doi.org/10.1016/S0891-5849(98)00315-3. PMid:10381194.
- Roselló-Soto, E., Galanakis, C. M., Brnčić, M., Orlien, V., Trujillo, F. J., Mawson, R., Knoerzer, K., Tiwari, B. K., & Barba, F. J. (2015). Clean recovery of antioxidant compounds from plant foods by-products and algae assisted by ultrasounds processing modeling approaches to optimize processing conditions. *Trends in Food Science & Technology*, 42(2), 134-149. http://dx.doi.org/10.1016/j.tifs.2015.01.002.
- Roy, M. K., Takenaka, M., Isobe, S., & Tsushida, T. (2007). Antioxidant potential anti-proliferative activities and phenolic content in watersoluble fractions of some commonly consumed vegetables: effects of thermal treatment. *Food Chemistry*, 103(1), 106-114. http://dx.doi. org/10.1016/j.foodchem.2006.08.002.
- Ruch, R. J., Cheng, S. J., & Klaunig, J. E. (1989). Prevention of cytotoxicity and inhibition of intercellular communication by antioxidant catechins isolated from Chinese green tea. *Carcinogenesis*, 10(6), 1003-1008. http://dx.doi.org/10.1093/carcin/10.6.1003. PMid:2470525.
- Sarmiento, A., Diaz-Castro, J., Pulido-Moran, M., Kajarabille, N., Guisado, R., & J. Ochoa, J. (2016). Coenzyme Q10 supplementation and exercise in healthy humans: a systematic review. *Current Drug Metabolism*, 17(4), 345-358. http://dx.doi.org/10.2174/138920021 6666151103115654. PMid:26526835.
- Scalzo, R. L., Genna, A., Branca, F., Chedin, M., & Chassaigne, H. (2008). Anthocyanin composition of cauliflower (*Brassica oleracea* L. var. botrytis) and cabbage (*B. oleracea* L. var. capitata) and its stability in relation to thermal treatments. *Food Chemistry*, 107(1), 136-144. http://dx.doi.org/10.1016/j.foodchem.2007.07.072.
- Shultz, S. (2013). Broccoli. *Journal of Agricultural & Food Information*, 14(4), 282-289. http://dx.doi.org/10.1080/10496505.2013.833839.

- Sultana, B., Anwar, F., & Iqbal, S. (2008). Effect of different cooking methods on the antioxidant activity of some vegetables from Pakistan. *International Journal of Food Science & Technology*, 43(3), 560-567. http://dx.doi.org/10.1111/j.1365-2621.2006.01504.x.
- Updike, A. A., & Schwartz, S. J. (2003). Thermal processing of vegetables increases cis isomers of lutein and zeaxanthin. *Journal of Agricultural and Food Chemistry*, 51(21), 6184-6190. http://dx.doi.org/10.1021/jf030350f. PMid:14518942.
- Uusiku, N. P., Oelofse, A., Duodu, K. G., Bester, M. J., & Faber, M. (2010). Nutritional value of leafy vegetables of sub-Saharan Africa and their potential contribution to human health: A review. *Journal* of Food Composition and Analysis, 23(6), 499-509. http://dx.doi. org/10.1016/j.jfca.2010.05.002.
- Villiers, A., Venter, P., & Pasch, H. (2016). Recent advances and trends in the liquid-chromatography-mass spectrometry analysis of flavonoids. *Journal of Chromatography. A*, 1430, 16-78. http://dx.doi. org/10.1016/j.chroma.2015.11.077. PMid:26718188.
- Wang, Z., Kwan, M. L., Pratt, R., Roh, J. M., Kushi, L. H., Danforth, K. N., Zhang, Y., Ambrosone, C. B., & Tang, L. (2020). Effects of cooking methods on total isothiocyanate yield from cruciferous vegetables. *Food Science & Nutrition*, 8(10), 5673-5682. http://dx.doi. org/10.1002/fsn3.1836. PMid:33133569.
- Xu, F., Jin, X., Zhang, L., & Chen, X. D. (2017). Investigation on water status and distribution in broccoli and the effects of drying on water status using NMR and MRI methods. *Food Research International*, 96, 191-197. http://dx.doi.org/10.1016/j.foodres.2017.03.041. PMid:28528099.
- Xu, Y., Xiao, Y., Lagnika, C., Song, J., Li, D., Liu, C., Jiang, N., Zhang, M., & Duan, X. (2020). A comparative study of drying methods on physical characteristics nutritional properties and antioxidant capacity of broccoli. *Drying Technology*, 38(10), 1378-1388. http:// dx.doi.org/10.1080/07373937.2019.1656642.
- Zhang, D., & Hamauzu, Y. (2004). Phenolics ascorbic acid carotenoids and antioxidant activity of broccoli and their changes during conventional and microwave cooking. *Food Chemistry*, 88(4), 503-509. http://dx.doi.org/10.1016/j.foodchem.2004.01.065.
- Zhong, X., Dolan, K. D., & Almenar, E. (2015). Effect of steamable bag microwaving versus traditional cooking methods on nutritional preservation and physical properties of frozen vegetables: a case study on broccoli (*Brassica oleracea*). *Innovative Food Science & Emerging Technologies*, 31, 116-122. http://dx.doi.org/10.1016/j. ifset.2015.07.002.