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ANTIOXIDANTS IN FRESH AND COOKED BROCCOLI (*Brassica oleracea* var. Avenger) AND CAULIFLOWER (*Brassica oleracea* var. Alphina F1)

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Abstract

Vegetables represent one of the most important components of the human diet. Brassica vegetables have been shown a great antioxidant capacity due to the presence of a multitude of biochemical substances such as vitamins, compounds with phenolic structure and pigments. Also it is known that some of these nutrients have a positive impact on the human health, reducing the risk of cardiovascular disease, diabetes and some types of cancer. This study evaluates the influence of two processing methods (boiling and steaming) on the stability of phenolic compounds, ascorbic acid, carotenoids and chlorophyll and on the antioxidant activity in edible part of broccoli and cauliflower. Boiling treatments caused losses of total phenolic compounds for both analysed vegetables (16.3% and 25.2%), while steaming led to comparable values with the fresh sample for broccoli and an increase by 12% for cauliflower. Thermal treatments determined a substantial loss of ascorbic acid for the analysed vegetables, ranging between 28% and 43.9%. Total carotenoid and chlorophyll content was higher in cooked samples and the antioxidant activity was lower after boiling and comparable with fresh vegetables when steaming.

***Key words**: antioxidant capacity, boiling, broccoli, cauliflower, steaming*

INTRODUCTION

The breathing process and various other reactions taking place in the human body result in formation of free radicals. In recent years, specialized research has focused on these reactive forms of oxygen, as they cause cell damage, leading to cancer, inflammation or adverse changes in blood vessels. In this respect, there has been increased interest in food substances that are capable of inhibiting or diminishing the harmful effects of free radicals in the human body (Sikora et al., 2008).
Several organizations such as European Food Safety Authority (EFSA), Food and Agriculture Organization (FAO), United States Department of Agriculture (USDA), World Health Organization (WHO) recommend high consumption of fruits and vegetables (Allende et al., 2006) because they offer protection against diseases, such as cardiovascular disease, cancer and cataract (Del Caro et al., 2004).
The contribution of the *Brassica* vegetables (e.g. broccoli, cauliflower, cabbage, kale) to health may be related to the antioxidant capacity of these vegetables due to the presence of phenolic compounds, carotenoids, vitamins and minerals. It is known that vegetables with antioxidant properties are rich in phenolics, especially phenolic acids and flavonoids. Research has shown that carotenoids possess several biological functions, which are associated with reduced risk of degenerative diseases, cataract prevention, reduced incidence of macular degeneration caused by aging, and reduced incidence of coronary heart disease (Krinsky, 1994; Podsedek, 2007; Gulçin, 2012).
Broccoli (*Brassica oleracea* var. italica) is part of the Cruciferous family (Podsędek, 2007). Broccoli contains a lot of nutritional substances

as glucosinolates, flavonoids, cinnamic acid derivatives, carotenoids, ascorbic acid and minerals. Broccoli contributes to health benefits due to its anticarcinogenic, antimutagenic and antioxidant properties. (Latté et al., 2011).

Cauliflower (*Brassica oleracea* L. var. botrytis) is also a member of the Cruciferous family. The main substances of cauliflower include glucosinolates, ascorbic acid, carotenoids, phenolic compounds and vitamin E. Also, it has anticarcinogenic and antioxidant effects, like other cruciferous vegetables (Llorach et al., 2003; Costache et al., 2012).

Before eating, most of vegetables need to be cooked to enhance their palatability and taste. However, cooking brings several physical and chemical changes in the composition of vegetables (Rehman et al., 2003; Balan, 2016). These changes could be both beneficial and detrimental depending on the extent and the type of the treatment conditions. Variety of effects like destruction, release and structural transformation of the phytochemicals take place during the cooking process. Cooking treatments like boiling, microwaving (Zhang & Hamauzu, 2004), baking, frying and griddling lead to changes in texture and nutritional properties of the vegetables.

This article attempts to investigate the influence of two cooking methods (boiling and steaming) on the stability of phenolic compounds, vitamins, pigments and on the antioxidant activity. The optimum cooking method was determined.

MATERIALS AND METHODS

Materials

The freshly harvested vegetables: broccoli (*Brassica oleracea* var. Avenger) and cauliflower (*Brassica oleracea* var. Alphina F1) were obtained from Development and Research Station for Vegetables Growing from Buzau. Chemicals like Trolox, DPPH and 2,6 diclorphenol-indophenol were purchased from Sigma-Aldrich Chemical CO. Meta-phosphoric acid, ethylenediaminetetraacetic acid, sodium hydrogen carbonate and sodium carbonate were purchased from Roth. Folin Ciocalteau reagent, ascorbic acid and analytical grade organic solvents (methanol and acetone) were purchased from Merck.

Methods

Sample preparation

The heads of broccoli and cauliflower were selected considering the absence of any damages and infections. The inedible parts were removed, then the heads were washed in tap water and then cut into pieces. After homogenization, three samples were obtained from each vegetable: one was retained raw as control and two were prepared for thermal processing (one for boiling and one for steaming).

Processing methods

The two types of cooking the vegetables were selected according to the incidence in Romanian cuisine. Both treatments, boiling and steaming, were conducted to obtain similar tenderness and taste (well done but firm when bitten). All the cooking variants were conducted in triplicates for each vegetable.

Boiling: Samples of broccoli and cauliflower were immersed in boiling water (1:4 vegetables/water), in a stainless-steel vessel, at 100°C temperature and boiled for 5 minutes. After boiling, the samples were drained off and quickly cooled.

Steaming: Samples of fresh broccoli and cauliflower were placed in a steamer vessel (Food Steamer Tefal) with 250 ml water, for 20 minutes (broccoli) and 10 minutes (cauliflower), at atmospheric pressure. After steaming, the samples were quickly cooled.

Analytical methods

For evaluating the influence of different processing methods (boiling and steaming) on the antioxidant properties of broccoli and cauliflower, quantification of total phenolics, ascorbic acid, carotenoids, chlorophylls and antioxidant activity were performed as follows.

Determination of total phenolic content

The total phenolic content of fresh and cooked broccoli and cauliflower was determined using Folin-Ciocalteau reagent (Singleton & Rossi, 1965). Briefly, 1 ml of 50% (v/v) methanolic extract was treated with 5 ml Folin-Ciocalteau reagent and 4 ml sodium carbonate (7.5% w/v). After 20 minutes keeping in the dark, the blue-coloured compound was measured spectrophotometrically at 752 nm with a Specord 210 UV-VIS spectrophotometer (Analytic Jena, Germany). Phenolics content was calculated using a calibration curve obtained with gallic acid. The results were expressed as mg GAE (gallic acid equivalents)/ 100 g dry weight (DW).

Determination of ascorbic acid

For quantification of ascorbic acid, the dye-titration method was used, according to AOAC procedure, 2000. Metaphosphoric acid extracts of uncooked and processed vegetables were subjected to titration with 2,6-dichlorophenolindophenol. In this oxidation-reduction reaction, the ascorbic acid from the extract was oxidized to dehydroascorbic acid and the indophenol dye was reduced to a colourless compound. The end point of the titration was detected when excess of the unreduced dye gave a rose pink colour in acid solution. Dehydroascorbic acid was not analysed in this study. The results were expressed in mg ascorbic acid/100 g dry weight.

Determination of pigments

Carotenoids and chlorophyll were extracted using a solvent mixture of acetone/water (80:20, v/v/). For a more efficient extraction, the vegetable sample mixed with the solvent was vortexed for 15 min, at 2000 rpm, at 20°C and then separated by centrifugation for 15 min, at 3500 rpm, at 20°C. The clear supernatant was further used for absorbance determination at 470, 646, 663 nm with Specord 210 UV-VIS spectrophotometer (Analytic Jena, Germany), as described by Lichtenthaler (1987). Using the specified equations, the results were expressed in mg/ 100 g dry weight.

Determination of antioxidant activity

The antioxidant activity was determined based on the reduction of 2,2-diphenyl-1-picryl-hydrazyl (DPPH) radical. A modified protocol was used (Culetu et al., 2016) consisting in extraction of the samples in methanol:water (1:1, v/v). After centrifugation (15 min, 3500 rpm, 20°C), 1 ml of supernatant was treated with 6 ml DPPH solution.

The reaction was carried out for 30 minutes in the dark and then the decline in absorbance was measured spectrophotometrically at 517 nm with Specord 210 UV-VIS spectrophotometer (Analytic Jena, Germany). The results were expressed in µmol Trolox/100 g dry weight.

RESULTS AND DISCUSSIONS

The processing methods used in this study had different effects on the values of antioxidant compounds (phenolics, ascorbic acid, chlorophyll and carotenoids) and on the level of antioxidant activity of the selected vegetables, as shown in Tables 1 and 2. The results obtained for all treatments were compared with those registered for fresh vegetables.

1. Total phenolic content

Phenolics represent a large group of metabolites which are present, in smaller or larger quantities, in plants. The amount of total phenolics in raw broccoli was 356.06 mg GAE/100 g DW. Researchers reported in broccoli different levels of total phenolic content ranging between 99.8 and 1204 mg GAE/100 g DW (Podsedek, 2007; Miglio et al., 2008; Ramos dos Reis et al., 2015). The big difference in the reported data may be due to the variation of the phenolic compounds according to the cultivars, agronomic conditions, harvesting stage, extraction solvent and the details of the analysis method. Thermal treatment of broccoli resulted in a loss of phenolic compounds when boiling (Table 1). The obtained value (297.97 mg GAE/100 g DW) is 16% lower than the one for fresh broccoli. The results are consistent with other works that reported the reducing of total phenolic content in broccoli up to 72%, according to the cooking time period (5-20 minutes) and also to the vegetable/water ratio (Zhang & Hamauzu, 2004; Porter, 2012; Danowska-Oziewicz et al., 2020). Steaming process didn't affect the level of total phenolics, the obtained value being comparable to the level recorded for the fresh sample (Table 1). The data reported in different studies showed a decrease of the total phenolics by up to 38% (Miglio et al., 2008) or even an increase by 18% comparing with untreated sample (Turkmen et al., 2004). When boiling, the temperature may enhance the extraction, but in the same time, the loss of biologically active compounds occurs, based on dissolution of polyphenols in water, depending on the type of vegetables and on the cooking time period.

Analysis of the fresh cauliflower revealed a total phenolics level of 381.49 mg GAE/100g DW. The reported data show different levels of phenolics, with values ranging between 27.8 and 782 mg GAE/100g DW, according to the used extraction solvent and the procedure (Podsedek, 2007; Hwang, 2019). Boiling the cauliflower sample for 5 minutes led to a 25.2% decrease in total phenolic content (Table 2). Steaming was

found to have a good effect on cauliflower, the obtained level for phenolics being 12% higher than the one of the uncooked sample.
The results are consistent with other studies that indicate an increase of the phenolic content for steamed vegetables, but there are others that reported lower levels (Girgin & Nehir, 2015; Hwang, 2019).
In their study, Vallejo et al. (2002) showed that a possible explanation for higher values of the steamed samples is the inactivation of oxidative enzymes due to the temperature and the lack of water. Another assumption is that the level of flavonoids is increased by the heating treatment due to their enhanced extractability (Stewart et al., 2000).

Table 1. Effect of cooking on antioxidant compounds and antioxidant activity of broccoli

	Fresh	Boiled	Steamed
Total phenolic content (mg GAE/100 g)	356.06±9.71	297.97±19.86	357.18±23.44
Ascorbic acid (mg/100 g)	600.32±14.34	368.94±18.25	391.45±20.11
Total chlorophyll (mg/100 g)	28.45±3.54	32.70±5.72	27.67±4.15
Chlorophyll a (mg/100 g)	22.71±3.08	27.88±5.14	22.5±3.66
Chlorophyll b (mg/100 g)	5.74±0.46	4.82±0.58	5.17±0.49
Total carotenoids (mg/100 g)	7.06±0.52	8.34±0.77	7.75±0.47
DPPH (µmol Trolox/100 g)	3420.18±94.15	2943.32±110.33	3251.74±106.28

*All values were reported at dry weight

**Values are presented as means ± SD of triplicate experiments

Table 2. Effect of cooking on antioxidant compounds and antioxidant activity of cauliflower

	Fresh	Boiled	Steamed
Total phenolic content (mg GAE/100 g)	381.49±12.50	284.94±20.12	426.43±16.59
Ascorbic acid (mg/100 g)	870.55±22.73	488.15±34.18	627.12±38.64
Total chlorophyll (mg/100 g)	nd	Nd	Nd
Chlorophyll a (mg/100 g)	nd	Nd	Nd
Chlorophyll b (mg/100 g)	nd	Nd	Nd
Total carotenoids (mg/100 g)	0.92±0.08	0.96±0.11	0.97±0.08
DPPH (µmol Trolox/100 g)	3455.04±71.43	2859.32±123.17	3401.61±99.86

*All values were reported at dry weight

**Values are presented as means ± SD of triplicate experiments

Among the two cooking methods performed in this study, boiling caused a decrease of the total phenolic content, while steaming had an increase effect.

2. Ascorbic acid

Vitamin C, in addition to being a nutrient, represents one of the most important antioxidant because it protects the body from free radicals. Because this vitamin is very sensitive at increased temperature, it is often used in research studies for evaluating the influence of different processing methods on vitamin content. The results of this study showed the effect of two cooking techniques on the level of ascorbic acid in broccoli and cauliflower. Fresh broccoli and fresh cauliflower showed the highest levels of vitamin C: 600.32 mg/100 g DW, respectively 870.55 mg/100 g DW. The performed cooking treatments (boiling and steaming) led to a significant decrease in the content of ascorbic acid (Tables 1 and 2). The highest loss was observed for both vegetables when boiling, the obtained results being by 38.55% and 44.1% lower than those recorded for the uncooked samples. This is probably mainly due to thermal degradation of ascorbic acid, but considering also water leaching (Lee & Kader, 2000). The retention of vitamin C during steaming processing was 65.2% for broccoli and 72.1% for cauliflower. These results are comparable with those reported by other researchers (Vallejo et al., 2002; Miglio et al., 2008). Although some studies reported a higher retention of ascorbic acid after the steaming process of broccoli (up to 74%), probably based on the reduced contact with water during thermal treatment, in the present study the extended cooking time (15 minutes) led to slightly lower results. Therefore optimal retention of vitamin C may be obtained with a thermal treatment using lower quantities of water when applied for shorter time periods.

3. Chlorophyll

An important parameter for assessing the quality of the cooked vegetables, like broccoli, asparagus and other leafy vegetables, is the degree of greenness. The green colour of the vegetables is given by chlorophyll pigments. These phytochemicals (chlorophyll a and chlorophyll b) are affected by heat treatments, during which they are transformed into other compounds, like olive-coloured pheophytins. In the present study the amount of chlorophyll a and b determined in fresh broccoli are comparable with those reported in other studies (Table 1). The performed cooking methods influenced the chlorophyll content in different manner. Chlorophyll a showed a 28.8% increased value for boiled broccoli. Danowska-Oziewicz et al. (2020) explained the higher value of chlorophyll a in boiled samples by a stronger interaction of the water with the plant tissue due to a better access. Regarding steaming processing, the contents of chlorophyll a and b were not significantly influenced (Table 1). These results are consistent with other published data (Ramos dos Reis, 2015; Danowska-Oziewicz et al., 2020). Mitic et al. (2013) reported a decrease of the chlorophyll a and b content in boiled broccoli.

4. Carotenoids

Carotenoids are important secondary plant metabolites that poses biological properties. Carotenes and their oxygenated derivatives act as antioxidants, scavenging and inactivating free radicals. Due to their chemical structure, these lipophilic compounds are sensitive to extreme temperature and light exposure, but also to the enzymes, metals and other oxidants action. Because during vegetables cooking, these factors can occur, it is important to assess the recovery of carotenoids after processing.

The results presented in Tables 1 and 2 show higher amount of total carotenoids in fresh broccoli compared with fresh cauliflower (7.06 mg/100 g DW and 0.92 mg/100 g DW, respectively). Boiling of broccoli sample resulted in 18.1% increase of total carotenoids, while steaming led to an only 9.8% increase comparing with the non-processed vegetable. These results are similar to those obtained by Ramos dos Reis et al. (2015) and Miglio et al. (2008), the reported retention of carotenoids ranging between 103-131% for boiling and between 119-131% for steaming. In their study Zhang & Hamauzu (2004) found that boiling broccoli led to the decrease of total carotenoids by 13% of the value registered for fresh sample, while steaming didn't affect significantly the carotenoids retention.

When cooking cauliflower by boiling and by steaming, the amount of carotenoids was slightly increased (by 4.3% and 5.4%, respectively) comparing with the level of fresh sample.

The obtained results confirm that carotenoids are not altered to a great extent by thermal treatments with short time periods and more, their content is increased probably due to greater chemical extractability and enzymatic degradation (Podsedek, 2005). Miglio et al. (2008), accounted for the improved extractability in boiled and steamed broccoli the disruption of carotenoid-protein complexes. When analysing the main carotenoids from fresh, boiled and steamed samples, Ramos dos Reis et al. (2015) found out that lutein and cryptoxanthin content was significantly increased in both broccoli and cauliflower, while β-carotene only in broccoli. The levels of zeaxanthin and α-carotene were lower for both cooked vegetables.

5. Antioxidant activity

Antioxidant activity is considered as an indicator of the overall benefits of the antioxidant compounds of a matrix. Fresh and cooked broccoli and cauliflower were tested for their hydrophilic antioxidant activity measured by DPPH radical scavenging method and the results are presented in Tables 1 and 2. The values of the antioxidant activity recorded for fresh studied vegetables didn't differ too much (3420.18 μmol Trolox/100 g DW for broccoli and 3455.04 μmol Trolox/100 g DW for cauliflower), being similar to those reported by Li et al. (2018).

The two performed cooking methods resulted in a slightly decrease of the antioxidant activity.

Boiling broccoli led to lowering it by 14%, while steaming only by 5.1% (Table 1). When cooking cauliflower, the loss of antioxidant activity was 17.2% for boiling and 1.5% for steaming (Table 2). Antioxidant activity was significantly correlated with total phenolic content (r^2 = 0.788) and moderate correlated with ascorbic acid content (r^2 = 0.474). Other research studies reported the same behaviour when boiling or steaming, but the decreasing of the antioxidant activity varies within wide limits, depending on the oxidation of the antioxidant compounds and their leakage in water (Zhang & Hamazu, 2004; Porter, 2012). In contrast with these findings, Ramos dos Reis et al. (2015) showed an improvement of antioxidant activity after such cooking methods due to thermal destruction of cellular walls and inactivation of prooxidant enzymes. The contradictory reported data are influenced by process parameters (extent of the cooking time period, temperature, and light exposure), variety and quality parameters of raw vegetables and the analytical methods.

CONCLUSIONS

The results of this study showed modification of the content of bioactive compounds and antioxidant capacity of broccoli and cauliflower subjected to boiling and steaming. After boiling the vegetables retained lower phenolics and ascorbic acid, while total chlorophyll and carotenoid content increased. Steam treatment resulted in better retention of antioxidant compounds (except ascorbic acid), with higher results for cauliflower. Antioxidant potential was lower after boiling and comparable with fresh broccoli and cauliflower when steaming. Accordingly, it may be stated that steaming is a better processing method because it minimises the loss of antioxidant properties of the tested vegetables, mainly due to the absence of water, but maintaining the advantage of temperature on improving extractability and oxidative enzymes inactivation.

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