

## Effect of Cooking on the Phenolic Content and Antioxidant Activity of Selected Vegetables Produced and Consumed in Cameroon

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### Abstract

This study was conducted in order to evaluate the effect of cooking time on the phenolic content and antioxidant activity of some vegetable: Carrots (*Daucus carota*), Green beans (*Phaseolus vulgaris*) and Green pepper (*Capsicum annuum*). The plant materials were each divided into four respective groups amongst which the first was boiled for 5 min, the second for 10 min, the third for 15 min and the fourth served as control (0 min). After all treatments, the vegetables were dried in the oven and used for the extraction of phenolic compounds and evaluation of the antioxidant activity. Results showed that boiling time significantly decrease ( $p<0.05$ ) the polyphenol content and the antioxidant activity of these vegetables. These vegetable should be eaten raw or cooked for a maximum duration of 5 min.

## Introduction

Natural antioxidants such as phenolic compounds, vitamin C and others are widely distributed in foods and vegetables. Many works have indicated that frequent consumption of foods rich in phenolic antioxidants such as fruits, vegetables, herbs, spices etc are associated in the reduction of the prevalence of cardiovascular diseases, neuro-degenerative diseases, cancers, aging... [1]. The beneficial role of natural antioxidants such as phenolic compounds, vitamin C and E has been attributed to their good antioxidant activity and protective effect against degenerative diseases [2]. Many of these antioxidants are also found in vegetables such as carrots, green beans and green pepper, which are significantly produced and consumed in Cameroon. They are generally used in the preparation of foods, and the famous processing method used is boiling. Vegetables processing such as boiling, blanching, microwaving, steaming and freezing are expected to affect the composition and availability of natural antioxidants [3]. During processing, the amount natural antioxidants such as heat labile phenolic compounds and vitamin C can significantly decrease, leading to a reduction of their antioxidant power [4]. The decrease in phenolic compounds in plant materials generally leads to a significant reduction of their protective action on humans, thus exposing them to several free-radicals-related disorders. Many studies have reported the effect of processing on the phenolic content and antioxidant activity of vegetables [2,5]. Zhang and Hamauzu (2004) demonstrated that cooking affect the antioxidant component and antioxidant activity of Broccoli [6]. Ismail et al. (2004) also showed that thermal treatments reduce the total phenolic content in all vegetables [7]. Though considerable attention had been given to the study of the effect of cooking on the phenolic content and antioxidant activity of vegetables, very few have been reported on the effect of cooking time on the phenolic content and antioxidant activity of vegetables produced and consumed in Cameroon, such as carrots, green pepper and green beans. Boiling time can significantly reduce the phenolic content and antioxidant activity of vegetables, thus reducing their protective effect against diseases due to oxidative stress.

The objective of this study is to evaluate the effect of boiling time on the phenolic content and antioxidant activity of three vegetables (Carrots, Green pepper and Green beans) produced and consumed in Cameroon.

## Materials and Methods

### Material

Fresh Carrots, Green beans and Green pepper (*Daucus carota*, *Phaseolus vulgaris*, *Capsicum annuum*) respectively were purchased from Muea local market, South-West Region, Cameroon in December 2017. All chemicals and reagents used were of analytical reagent grade.

### Methods

#### *Sample Preparation and Processing*

Carrots, Green bean and Green pepper were cleaned and divided into 4 groups of 500 g each. Carrot, Green beans and Green pepper were respectively divided into 04 groups, which were respectively boiled in 1 L of water at 98°C for 5, 10 and 15 min. The last groups for each vegetable was not processed and served as control.

It is important to note that the vegetables were not in direct contact with water during cooking. After this, all the above mentioned samples were dried in an electric oven for 48h at 50°C before being used for further analysis.

### ***Extraction of Vegetable Polyphenols***

Dried vegetables samples were grounded using a grinding machine (Moulinex). 20 g of each sample power was extracted with 400 ml of methanol for 48h at room temperature. The mixture was regularly subjected to shaking during the extraction. The extract was filtered with a Whatman No. 1 filter paper, and residue was again extracted with 200 ml of methanol to ensure maximum extraction of phenolic compounds. The combined filtrates were subjected to rotary evaporation at 40°C under reduced pressure for the removal of the solvent. The dried extract was used for the determination of the total phenolic content and antioxidant activity.

### ***Effect of Processing on the Antioxidant Activity of Vegetables***

#### ***Radical Scavenging Activity***

The radical scavenging activity of each extract was determined using the 2,2-diphenyl-1-picryl hydrazyl (DPPH) method, as described by Womeni *et al.* (2016) [9]. A total of 4.5 ml of 0.002% alcoholic solution of 2,2-diphenyl-1-picryl hydrazyl (DPPH) was added to 0.5 ml of different concentrations (250, 500, 1000, and 2000 µg/ml) of samples and standard solutions separately, in order to have final concentrations of products of 25-200 µg/ml. The samples were kept at room temperature in the dark and after 30 min and the absorbance of the resulting solution was measured at 517 nm. The absorbance of the samples, control, and blank was measured in comparison with methanol. The antioxidant activity (AA) was calculated according to the formula:

$$\text{AA\%} = [( \text{Abs}_{\text{control}} - \text{Abs}_{\text{sample}} ) \times 100 / \text{Abs}_{\text{control}} ]$$

AA = Antioxidant activity, Abs<sub>control</sub> = Absorbance of the DPPH solution, Abs = Absorbance of the sample

#### ***Ferric Reducing Antioxidant Power***

The antioxidant potential of carrot, green pepper and beans extracts was also evaluated by their ability to reduce iron (III) to iron (II) following the method of Oyaizu (1986). An aliquot of 0.5 mL plant extract (250, 500, 1000, and 2000 µg/ mL) was mixed with 1 mL phosphate buffer (0.2 M, pH 6.6) and 1 mL of 1% aqueous K<sub>3</sub>Fe(CN)<sub>6</sub> solution, well shaken and incubated at 50°C for 30 min. After incubation, 1 mL of 10% TCA solution was added to stop the reaction and the mixture was centrifuged at 1008 g for 10 min. 1.5 mL of supernatant, 1.5 mL of distilled water and 0.1 mL of 0.1% FeCl<sub>3</sub> solution were mixed and incubated for 10 min and absorbance read at 700 nm on spectrophotometer.

The final concentration of the extract solutions were of 12.5-200 µg/mL. A sample blank, containing all the reagents but no extract was prepared in the same conditions. A higher absorbance indicates a higher reducing power.

## Statistical Analysis

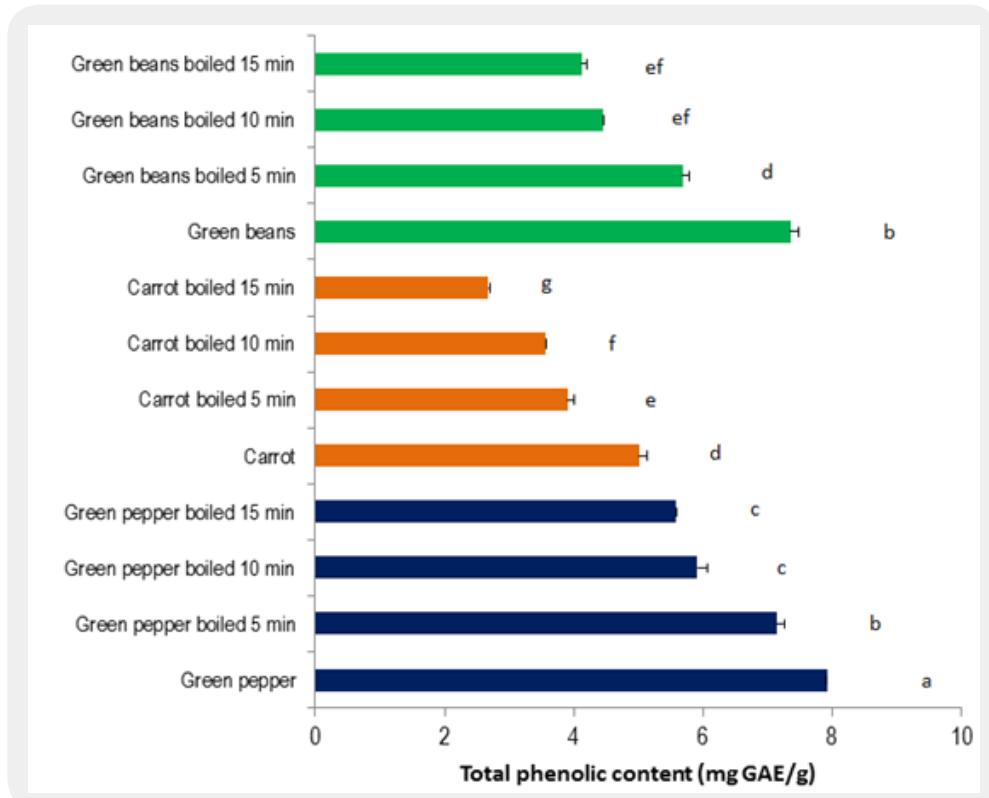
Results (Mean±Standard deviation) obtained in the present study were subjected to one-way analysis of variance (ANOVA) with Student-Newman-Keuls test using Graphpad-InStat version 3.05, to evaluate the statistical significance of the data. A probability value at  $p<0.05$  was considered statistically significant.

## Results and Discussion

### Results

#### Total Phenolic Content

The change in total phenolic content of Carrots, Green pepper and Green beans during processing is presented in Figure 1. A significant decrease ( $p<0.05$ ) in phenolic content was registered in all the samples compared to their respective controls. The highest phenolic content was recorded in Green pepper followed by the Green beans while the lowest one was registered with Carrots.

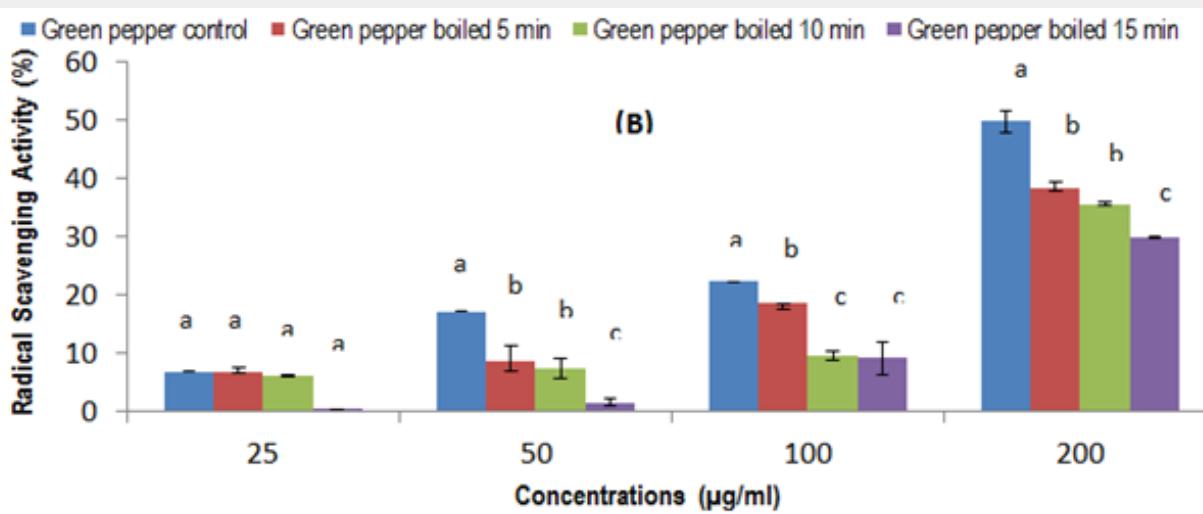
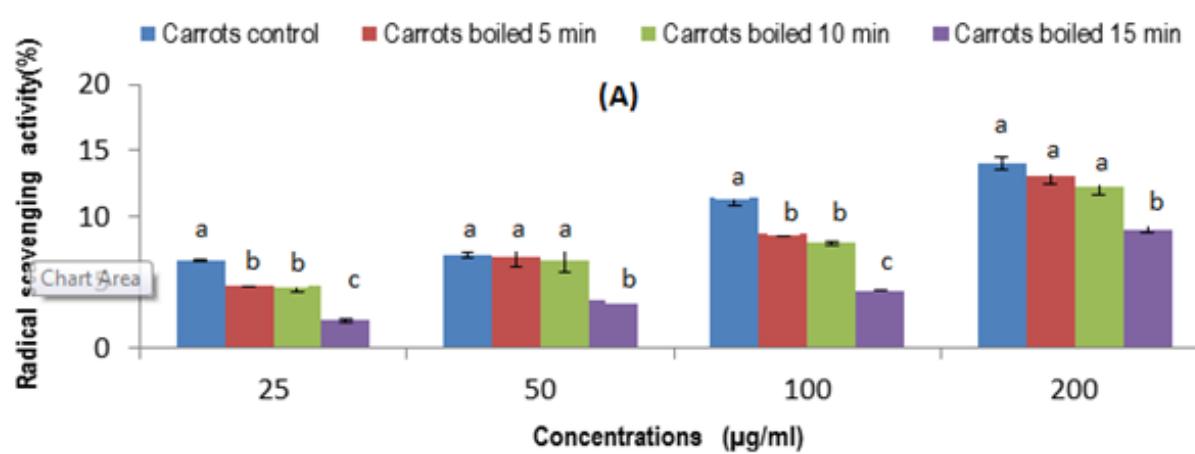


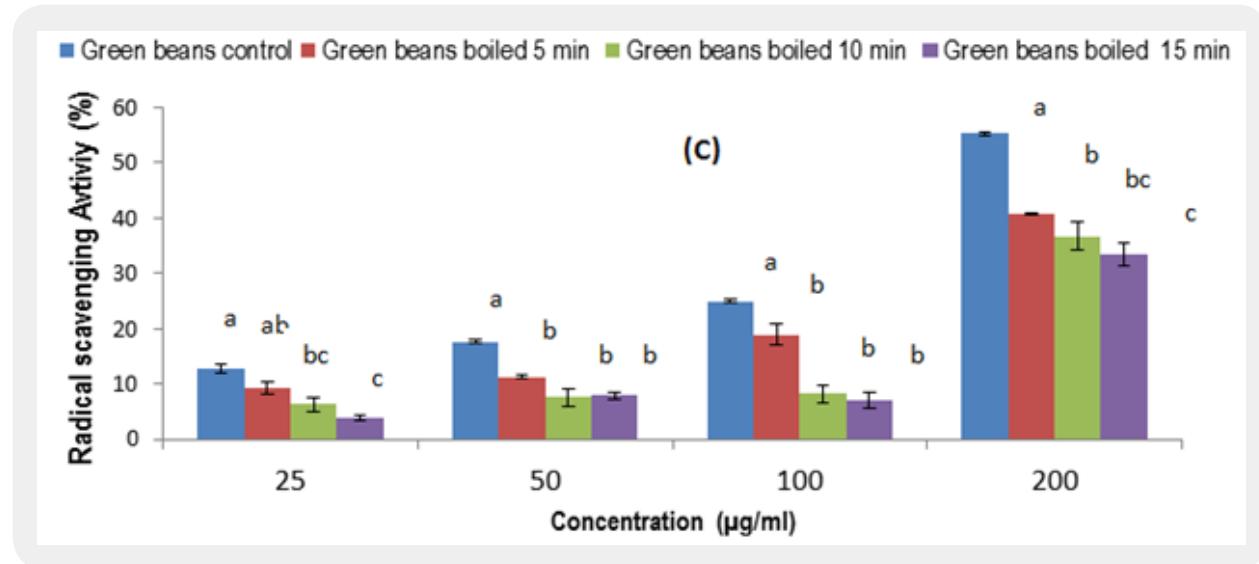
Values are presented as Mean±SD. <sup>(a-d)</sup>Values with different superscripts are significantly different ( $p<0.05$ )

**Figure 1:** Evolution of the total phenolic content of Carrot, Green Pepper and Green Beans during processing

### 2,2 -Diphenyl-1-Picryl Hydroxyl Test

The changes in radical scavenging activity of carrots, Green pepper and Green beans are presented in figure 2 (A-C). Generally as previously observed with the total phenolic content the radical scavenging activity of these vegetables was significantly decreasing ( $p<0.05$ ) with boiling time and this at all concentrations. Compared to the other extracts Carrots extracts have exhibited significantly lower DPPH radical scavenging activity. Its antioxidant activity varied from 4-14%, while that of Green beans and Green pepper was respectively ranged between 5-55% and 10-50%. However the activity of all these extracts was increasing with the concentration.



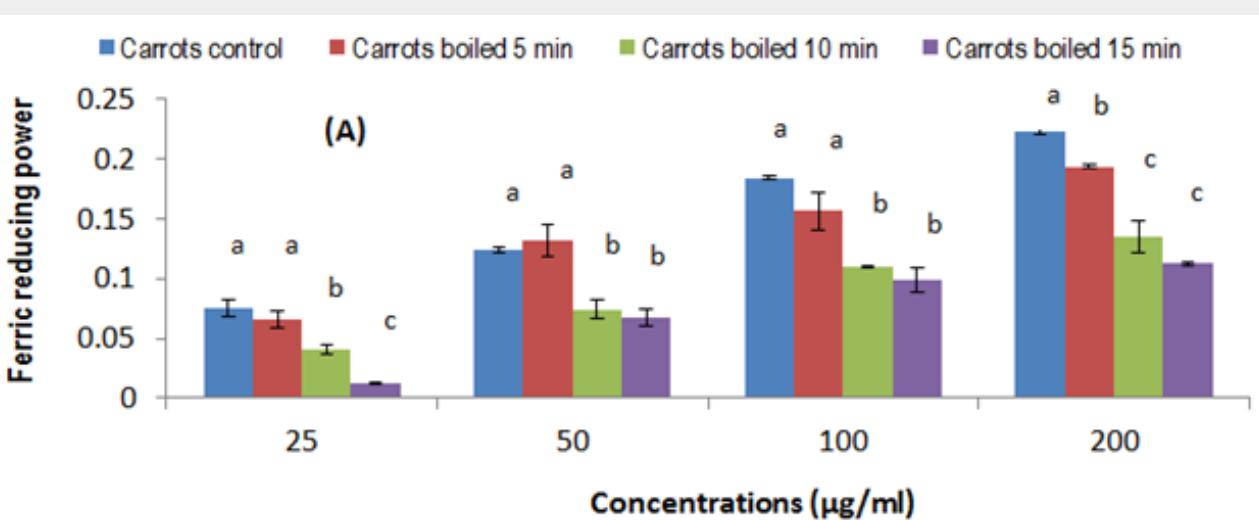


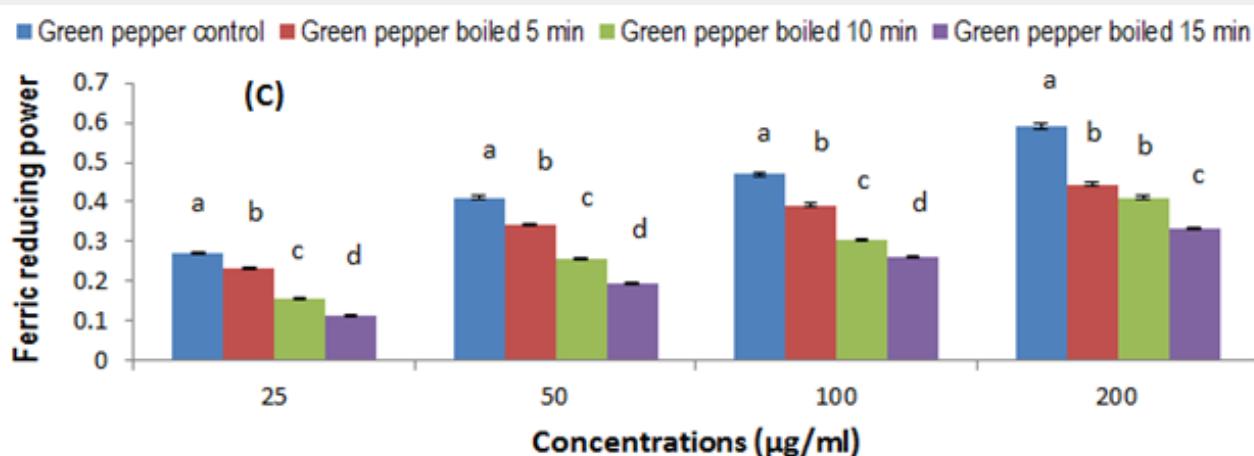
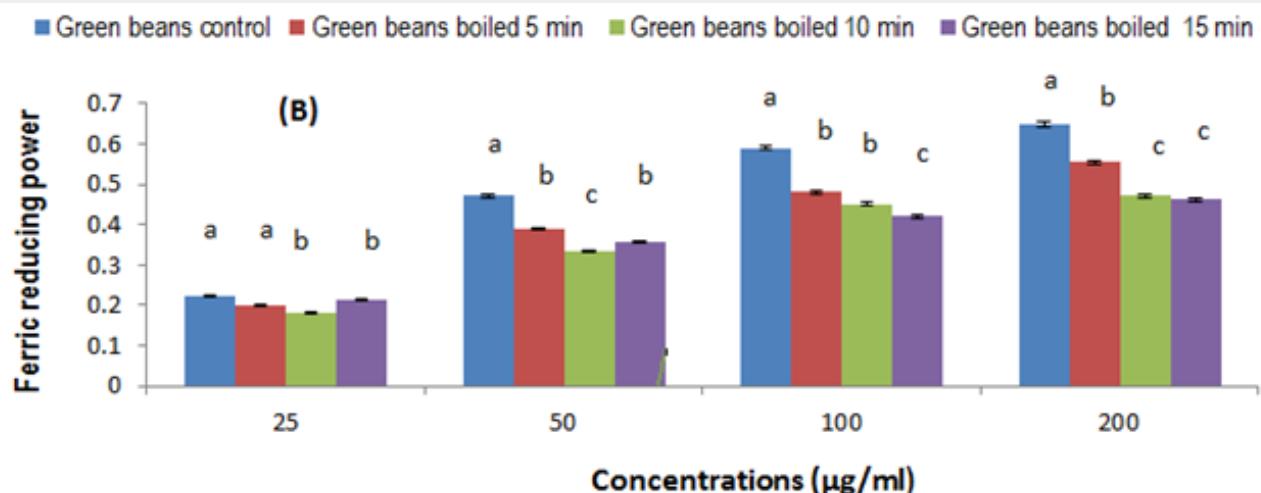
Values are presented as Mean $\pm$ SD. <sup>(a-d)</sup> Values with different superscripts at the same concentration are significantly different ( $p<0.05$ )

**Figure 2:** Changes in Radical scavenging activity of Carrots (A), Green pepper (B) and Green beans (C) during processing

#### Ferric Reducing Antioxidant Power

The variation in the ferric reducing antioxidant power of carrots, green pepper and green beans are presented in figure 3 (A-C). A significant decrease ( $p<0.05$ ) in antioxidant activity with boiling time was registered in all the samples and at each concentration compared to their respective controls. The ferric reducing antioxidant power of all the sample was increasing with their concentration the highest was recorded with Green pepper (0.15-0.59) followed by Green beans (0.22-0.6). The lowest activity was registered with Carrot extract (0.01-0.22).





Values are presented as Mean $\pm$ SD. <sup>(a-d)</sup> Values with different superscripts at the same concentration are significantly different ( $p<0.05$ )

**Figure 3:** Changes in Ferric reducing antioxidant power of Carrots (A), Green pepper (B) and Green beans (C) during processing

## Discussion

The evaluation of the total phenolic content of carrots, green pepper and green beans showed that, this parameter significantly decrease ( $P<0.05$ ) with boiling time and that green pepper had the highest phenolic content followed by green beans. The fact that the total phenolic content of vegetable decrease during processing has already been reported. Preti *et al.* (2017) have demonstrated that the total phenolic content of green beans significantly decrease after cooking by steaming and boiling [10].

In the same line Turkmen *et al.* (2005) showed that the phenolic content of some vegetables such as peas and leek significantly decrease after boiling, steaming and microwaving [5]. On the other hand, Shotorbani *et al.* (2012) showed that the phenolic content of sweet pepper also known as green pepper was decreasing with incubation time at high temperature [11]. These observations are in agreement with the results obtained in this study. However, in some reports it has been demonstrated that such treatments lead to an increase of the total phenolic content of some vegetables such as spinach, broccoli and pepper [5]. The reduction in total phenolic content observed in all the vegetables with boiling time can be attributed to the breakdown of these compounds during cooking at high temperature [12]. This process can be increased by the fact that prior to cooking the vegetables were cut to remove the unedible parts. Cutting operations have been reported to reduce the polyphenol concentration due to oxygen exposure and the activation of polyphenol oxidase [13,14].

The evaluation of the antioxidant activity of carrots, green pepper and green beans showed that their activity is proportional to their concentration, and significantly decrease ( $p<0.05$ ) with boiling time. The decrease in antioxidant activity of vegetable during processing is in correlation with the decrease in total phenolic content previously registered. In fact phenolic antioxidants are large class of plant secondary metabolite that have been proven to be responsible of their good antioxidant activity. Generally, plants with a high total phenolic content also exhibit high antioxidant activity [15,16]. The decrease in antioxidant activity of carrots, green pepper and green beans with boiling time observed in this study is in agreement with the results reported by Preti *et al.* (2017) who showed that the DPPH Radical Scavenging Activity of purple, green and yellow beans was significantly decreasing after boiling and steaming [10]. However, these results are not in accordance with those reported by Bembem and Sadana (2014) and Turkmen *et al.* (2005) who showed that the antioxidant activity of pepper, squash, green beans, broccoli, spinach and carrots was increasing with boiling and steaming [17,5]. The cooking techniques and conditions might be responsible for the changes observed. In order to benefit from the phenolic antioxidants present in these vegetables short cooking time (5 min) is preferable.

## Conclusion

The objective of this study was to evaluate the effect of cooking by steaming on the phenolic content and antioxidant activity of carrots, green beans and green pepper. Results showed that the total phenolic content and antioxidant activity of these vegetables significantly decrease with boiling time. Overcooking these vegetables is not suitable as it leads to the loss of natural phenolic antioxidants which can help in reducing the prevalence to diseases related to oxidative stress. These vegetables should not be cooked for more than five minutes by steaming.

## Conflict of Interest

Authors have declared that no competing interests exist.

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