THE EFFECTS OF COOKING METHODS ON ANTIOXIDANT ACTIVITY AND PHENOL CONTENT IN VEGETABLES

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ABSTRACT

Introduction: Total phenol and antioxidant activity of vegetables may be changed by different ways of cooking such as, baking, steaming, pressure cooking, microwaving and frying. This study is conducted to determine the antioxidant activity and phenol content of three Iranian vegetables by different cooking methods. Materials and Method: Fresh Broccoli (Brassica oleracea, L. pepper (Capsium annuum, L and spinach (Spinacia oleracea,L) were obtained from a market in Yasuj, Iran. One portion was kept raw as control and stored at 4 °C in the refrigerator, others were subjected for four different cooking methods. After cooking, the cooked vegetables were cooled and then all vegetables were homogenized in a 2 min , and stored at −20 °C until analyses. Raw and cooked vegetables were analyzed by different antioxidant activity methods and total phenol contents based on dry matter. Results: The fresh vegetables total phenol content ranged 882–1306 mg GAE/100 g dm. After cooking procedures, the total phenolics content of spinach and broccoli was insignificantly reduced. Conversely, total phenolic content of pepper was significantly increased in boiling (8%) and frying (4%) methods. Total antioxidant activity of DPPH against of TEAC method in all samples increased during all cooking procedures compared to the fresh procedure. Conclusion: depends on cooking methods and kind of vegetables nutritional and physicochemical qualities changed by domestic cooking. Moreover, these results suggest
further studies are needed to identify a preferential cooking method for each vegetable to improve the nutritional quality and their reliability.

**Keywords**: Antioxidant Activity, *Brassica oleracea, Capsium annuum, Spinacia oleracea*, Cooking Methods.

**INTRODUCTION**

Fruit and vegetables are important sources of vital micronutrients and vitamins. They protect the human body from chronic disease including cardiovascular disease, immune dysfunction and some cancers [1]. It well documented the 4.4% of disease, disability and death is attributed to the low intake of fruit and vegetable [2]. So, the increase consumption of fruit and vegetables in children leads to protect of childhood disease [3]. In addition, vitamins, different minerals, various compounds such as flavonoids, isoflavones, flavones, anthocyanins and tocopherol in vegetables have chemopreventive and cardioprotective [4].

Therefore, the way a vegetable is prepared may be affects on its nutrient content. Various methods such as boiling, frying and pressure cooking of vegetables have different effect on their antioxidant activity [5]. Although, cooking killed the microbes of the vegetables and makes safer and increase flavor to eat them. But, it has been shown that overcooking of vegetables can be destroys vitamins and micronutrients and results in poor materials, appearance, and taste [6]. Furthermore, the steaming is the preferred method for cooking of green vegetables such as Broccoli, L. pepper and spinach because during steaming nutrients and flavor are more preserved and these vegetables does not break up [7]. Whereas, boiling is the common method of cooking vegetables but in most cases break up delicate vegetables and destroy nutrients. Today's, vegetables are prepared at home on the basis of flavor and taste preference rather than nutrient losses. Total phenol and antioxidant activity of vegetables may be changed by different ways for cooking such as, baking, steaming, pressure cooking, microwaving and frying [5]. There is evidence that the microwaving is better for nutrient preservation because it uses small water in a short time cooking. While, boiling vegetables in water leads a considerable loss of water-soluble vitamins and chemical composition of vegetables. Frying make better the flavor quality of vegetables by formation of aroma compound. Different frying methods including deep, roasting and shallow frying have varying effects on chemical compound and total phenol of vegetables. It has been documented that the content of minerals, proteins and carbohydrates are more preserved by
fraying than boiling [8]. However, there are less study about the antioxidant and phenolic content change of vegetable by different cooking methods. So this study is conducted to determine the antioxidant activity and phenol content of three Iranian vegetables by different cooking methods.

METHODS AND MATERIALS

SAMPLE PREPARATION

Fresh Broccoli (Brassica oleracea, L. pepper (Capsium annuum, L and spinach (Spinacia oleracea,L.) used in this research and obtained from a market in Yasuj, Iran and immediately cleaned by removed manually non edible parts with a sharp knife. All vegetables were carefully washed with water (in consumer conditions), dried air and were cut into almost equal small pieces, mixed well.

The vegetable samples (2500 g) was taken and divided into five portions (500 g each). One portion was kept raw as control and stored at 4 ◦C in the refrigerator in home consumer conditions, others were subjected for four thermally treatments in triplicate. The best cooking times were determined as previously described and according to common cooking techniques [9] which conducted by trained researchers (Table 1). Cooking conditions for each vegetable were examined, with a preliminary experiment in our laboratory.

Table 1. Different Cooking procedures with necessary times used to cook the vegetable.

<table>
<thead>
<tr>
<th>Cooking procedures</th>
<th>Boiling</th>
<th>Pressure-cooking</th>
<th>Microwave cooking</th>
<th>Frying</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vegetables</td>
<td>Times (min)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Broccoli</td>
<td>16</td>
<td>5</td>
<td>2</td>
<td>15</td>
</tr>
<tr>
<td>Green pepper</td>
<td>20</td>
<td>5</td>
<td>4.5</td>
<td>17</td>
</tr>
<tr>
<td>Spinach</td>
<td>13</td>
<td>3</td>
<td>2</td>
<td>12</td>
</tr>
</tbody>
</table>

Boiling: Vegetable (500 g) was added to 750 ml of water in a stainless steel pan that had just reached at 1000 C and cooked for 13-20 min. The samples were drained off and cooled in room temperature for a few minutes.

Microwave cooking: the vegetable (500 g) was placed in a glass dish which placed in the oven is heated by microwaves from all directions, and cooked in a commercial – 1000 W
microwave oven. Cooking took 2 min for spinach and 2 min for, broccoli, pepper. Samples were drained off and cooled in room temperature for a few minutes.

**Pressure-cooking:** vegetables (500 g) were placed in a pressure cooker (stainless steel, 22 cm diameter, MagefesaR, Zaragoza, Spain), containing water (300 mL) and a pressure valve for high pressure-cooking. In this method the temperature of boiling water can be raised above 100°C.

**Frying:** the vegetable sample (500 g) was placed in a heated metal or frying pan with hot refined olive oil (169 °C) (100 mL) without covering it, and stirred until the sample became crisp-tender.

After cooking, the cooked vegetables were cooled for a few minutes at room temperature and then all vegetables were homogenized in a blender (Moulinex-France) for 2 min, and stored in at −20 °C until analyses. All parameter were determined on dry matter basis due to various water content of sample. For prepared of the dry matter, 3–4 g of raw or cooked homogenized sample (as triplicate) was placed in a ceramic container dried in a oven at 70°C for at least 2 days until reaching constant weight. Raw and cooked vegetables were analyzed by different antioxidant activity methods and total phenol contents.

**Determination of total phenol**
The total phenolic contents of fruit extracts were estimated using the Folin-Ciocalteau reagent technique with slight change. Total phenol was expressed as Gallic acid equivalent (GAE) /g extract [10].

**Antioxidant Activity of Diphenypicrylhydrazyl (DPPH)**
Antioxidant capacity of fruit extracts determined with some modification. Percent of inhibition was estimated as follow: Inhibition % = [(A0 - A1)/A0] ×100, A0 is the absorbance of control and A1 is the absorbance of the plan extracts (10).

**Trolox equivalent antioxidant activity (TEAC):** The antioxidant activity was measured using TEAC, based on Arnao method with some modification [11]. Percent of inhibition same DPPH method was calculated.
Statistical Analysis
All data were expressed as means ± standard deviation of (n=3) measurements. For detect of significant differences in vegetable samples one-way analysis of variance (ANOVA) was applied which followed by post hoc tests. P-values less than 0.05 were considered significant.

RESULT
The fresh vegetables total phenol content ranged 882–1306 mg GAE/100g dm and the rankings were spinach > pepper > broccoli > (Table 2). After cooking procedures, the total phenolics content of spinach and broccoli was insignificantly reduced in spinach 12,11,11.7 and 4.5 % in boiling, Pressure-cooking Microwave cooking and frying cooking methods respectively (Table 2). Pattern of reductions in broccoli were 18, 13, 12.7 and 4.5 % in boiling, Pressure-cooking, frying and Microwave cooking methods respectively. Conversely, of pepper total phenolic content was significantly increased in boiling (8%) and frying (4%) methods. Although, a little decrease in total phenolics of pepper was also observed in frying (7.4%) and Microwave (7.6%) cooking methods (Figure 1).

Antioxidant activity of fresh vegetables as determined by the DPPH radical scavenging method decreased in the order: broccoli > spinach > pepper (Table 2). Among all these test vegetables broccoli showed highest scavenging activity with a inhibition of 87.7% whereas pepper had lowest activity with 68.7%.

Total antioxidant activity of broccoli, spinach and pepper increased during all cooking procedures compared to the fresh procedure. The increase in antioxidant activity of pepper and spinach was the almost same during all cooking procedures. However, antioxidant potential of broccoli was keep the same as for fresh value in each cooking procedures( Figure 2). TEAC radical scavenging capacity for raw and cooked vegetables expressed by TEAC value (Figure 3).

Antioxidant activity of fresh vegetables according to TEAC method decreased in the order: spinach> pepper > broccoli. In TEAC antioxidant activity against to DPPH antioxidant assay all vegetables broccoli, spinach and pepper decreased during all cooking procedures compared to the fresh procedure (Table 3,4). Pepper in boiling method in TEAC assay produced the lowest loses (7%) and highest losses were reported in broccoli (20%). In Pressure-cooking the lowest (7.9%) and highest losses (33%) was seen in pepper and broccoli.
respectively. In microwaving, the lowest (11.5%) and highest losses (17%) were seen in spinach and broccoli respectively.

In summary, according to analysis of the ABTS radical scavenging activity the highest losses were reported in broccoli in Pressure-cooking method (Figure 1).

Table 2. Percentage of change in total phenol by different cooking methods in 3 Iranian vegetables

<table>
<thead>
<tr>
<th>Vegetables</th>
<th>Boiling</th>
<th>Pressure-cooking</th>
<th>Microwave cooking</th>
<th>Frying</th>
</tr>
</thead>
<tbody>
<tr>
<td>Broccoli</td>
<td>↓18</td>
<td>↓13</td>
<td>↓4.5</td>
<td>↓12.7</td>
</tr>
<tr>
<td>Green pepper</td>
<td>↑8</td>
<td>↓7.4</td>
<td>↓7.6</td>
<td>↑4</td>
</tr>
<tr>
<td>Spinach</td>
<td>↓12</td>
<td>↓11</td>
<td>↓11.7</td>
<td>↓4.5</td>
</tr>
</tbody>
</table>

Table 3. Percentage of change in Antioxidant activity of DPPH by different cooking methods in 3 Iranian vegetables

<table>
<thead>
<tr>
<th>Vegetables</th>
<th>Boiling</th>
<th>Pressure-cooking</th>
<th>Microwave cooking</th>
<th>Frying</th>
</tr>
</thead>
<tbody>
<tr>
<td>Broccoli</td>
<td>↑2</td>
<td>↑1</td>
<td>↑2</td>
<td>↑4</td>
</tr>
<tr>
<td>Green pepper</td>
<td>↑12</td>
<td>↑11.6</td>
<td>↑12.3</td>
<td>↑12</td>
</tr>
<tr>
<td>Spinach</td>
<td>↑11.3</td>
<td>↑11.7</td>
<td>↑11.6</td>
<td>↑12.1</td>
</tr>
</tbody>
</table>

Table 4. Percentage of change in antioxidant activity of TEAC by different cooking methods in 3 Iranian vegetables

<table>
<thead>
<tr>
<th>Vegetables</th>
<th>Boiling</th>
<th>Pressure-cooking</th>
<th>Microwave cooking</th>
<th>Frying</th>
</tr>
</thead>
<tbody>
<tr>
<td>Broccoli</td>
<td>↓0</td>
<td>↓33</td>
<td>↓17</td>
<td>↓26</td>
</tr>
<tr>
<td>Green pepper</td>
<td>↓7</td>
<td>↓7.9</td>
<td>↓13.6</td>
<td>↓9</td>
</tr>
<tr>
<td>Spinach</td>
<td>↓13.8</td>
<td>↓11.5</td>
<td>↓11.5</td>
<td>↓15.3</td>
</tr>
</tbody>
</table>
Figure 1: Effect of different cooking methods on total phenolic content of vegetables

Figure 2: Effect of different cooking methods on antioxidant activity by DPPH method in vegetables

Figure 3: Effect of different cooking methods on antioxidant activity by TEAC in some vegetables. The extracts for antioxidant activity were tested at concentration of 10 mg/ml on dry basis.
DISCUSSION
Vegetables are a major source of antioxidants and it is needed to defend against the free radicals. Therefore, it is necessary to assess the antioxidant activity and total phenol content of the vegetables by different processing methods. Result of this study suggested that the percentage of the total phenol for the broccoli and spinach decreased by boiling compare to the microwave cooking and frying. Microwave cooking had a significant effect on increase of total phenol ($p < 0.05$) of broccoli compare to the boiling. While the other two methods caused a slight but not significant. Although in present study total phenolic content was significantly increased in boiling (8%) and frying (4%) methods, Buna fund that heat can break supramolecular structure and case releasing phenolic bounds which appears with the Folin–Ciocalteau reagent [12]. Although in this study each vegetables had different antioxidant activity depends on their various antioxidant components but, the antioxidant activity by TEAC decreased for three vegetables for all of the cooking methods. Further, this study showed the total phenol of the green pepper was slightly increased by boiling, and lost during frying. In accordance with current result, there is documented that the preserving of the β-carotene during boiling is better than the steaming, so this finding suggested the increase of temperature is the great factor that affecting the macronutrients and micronutrients stability compare to the boiling water [13].

It has been known the antioxidant activity of some vegetables such as kale, spinach and swamp cabbage was significantly reduced after one min of high thermal cooking [14]. Our result demonstrated in antioxidant activity by the DPPH method, broccoli had with highest scavenging activity with a inhibition of 87.7% and pepper had lowest activity with 68.7%. Furthermore, antioxidant activity of pepper and spinach was the almost same during all cooking procedures. In agreement with this result Turkmen reported the phenolic acids in some vegetables such as spinach is more in the outer layers and are easily exposed to the boil water that that causes the reduce the antioxidant activity [15]. As previously reported there were no significant differences in the antioxidant activity of the some vegetables by boiling and microwave cooking [16]. While, depend on cooking procedures the phenol of spinach, peas and leek were decreased due to breakdown of phenolics during cooking.

In this research the antioxidant activity of pepper, spinach and broccoli are more preserved by microwave than boiling and pressure cooking this result is agree with Yamaguchei study that showed microwave heating keep the active micronutrition in the cooked tissue [17].
Furthermore, total antioxidant activity of pepper, broccoli and spinach significant increases by microwave cooking and steaming [15].

This result suggested that the bioactive compound probably degraded during boiling water that leads to decrease of the active element. There are different approaches that used to ascertain the antioxidant activity so, in some study there was not relationship between the phenolic content and antioxidant activity [18].

Further, different phenolic elements may be having different reaction to the Folin-Ciocalteu method. Result of this study is agreement with Velioglu etal that demonstrated there was not relationship between the total phenolic and antioxidant activity in some vegetables with different methods cooking [19].

In recent study broccoli have high antioxidant activity by DPPH method whereas it has low total phenolic content. However, the antioxidant activity or scavenger capacity of vegetables are depended not only on the cooking method but also on their bioactive structures, cutting, chopping and stability of the structure to heat, and it is not clear which factor contributes to the increase of antioxidant activity [20].

CONCLUSION
The present study indicates depends on cooking methods and kind of vegetables nutritional and physicochemical qualities changed by domestic cooking. Moreover, these results suggest further studies are needed to identify a preferential cooking method for each vegetable to improve the nutritional quality and their reliability.

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REFERENCES


