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The effect of different cooking methods on antioxidant activity of fruits and vegetables

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Abstract

A diet rich in fruits and vegetables possess wide variety of antioxidant compounds that are very important for human health. The antioxidant capacity may be decreased or increased by thermal-processing. This study aimed to evaluate the antioxidant capacity by using DPPH method and by using different bacterial strains, *S. aureus* (Gram-positive bacteria) and *E. coli* (Gram-negative bacteria). Fresh vegetable and some fruit juices showed different values of scavenging activity. Pepper showed the highest scavenging activity. Under cooking conditions, 30 minutes of boiling, pomegranate, tomato, spinach and onion showed an increase in scavenging activity. Cooking in microwave for 15 minutes caused increase in scavenging activity for all fruits and vegetables except Pepper. Addition of vitamin C or fresh lemon juice to *E. coli* incubated with H_2O_2, protected the *E. coli* against the damaging effect of H_2O_2. In conclusion, processing of fruits and vegetables by boiling or by using microwave did not decrease antioxidant activity rather than, in some cases these cooking method increase the antioxidant activity.

1. Introduction

Reactive oxygen species (ROS) participate in many chemical reactions leading to damage in several molecular species including lipids, proteins, and nucleic acids and this can cause oxidative stress [1, 2].Oxidative stress can lead to hypertension, cancer, atherosclerosis, diabetes, Alzheimer, Parkinson and other diseases [3, 4]. Antioxidant are known as substances that can prevent oxidation process and delay or inhibit cellular damage due to their free radical scavenging property [5, 6].

Several health benefits can be achieved by consumption of a diet that is rich in fruits and vegetables. An alternative way to consume proper amounts of nutrients is to consume beverages such as juices. During the last few years, the demand for these beverages has been rising all over the world [7]. Such diet can provide not only essential nutrients but also phytochemicals that are very important for promoting health and preventing from diseases [8]. Consumption of fruits and vegetables is found to be strongly associated with reduction of risk of many common diseases such as cardiovascular disease, Alzheimer disease, diabetes, cancer, cataracts, and age-related functional decline [9, 10].

Many studies found that fruits and vegetables can supply a wide variety of antioxidant compounds that are very important for human health. Preventive effect of individual antioxidant in a dietary supplement is found to be less effective when it is taken through diet in fruits and vegetables [11]. Thus, the health-promoting effects of dietary fruits and vegetables probably reflects a complex interaction among many naturally occurring compounds, which has not been duplicated by consumption of isolated antioxidant compounds [7].

Several studies take into consideration the facts that, the concentration of natural antioxidant and their bioavailability may be changed by different cooking method like microwave and boiling [12, 13]. Thermal-processing causes the oxidation and other degradation reactions and these conditions might result in the loss of many natural antioxidant content like vitamins C and A. Because of the oxidation and degradation reactions, processed fruits and vegetables have been supposed to have a lower antioxidant activity than their respective freshly one [14-16].

There are many available methods that are known to be used in vitro to estimate and evaluate the antioxidant activities of natural sources of phytochemical compounds like fruits and vegetables [17, 18]. In this study the effect of microwave and boiling on the antioxidant capacity were evaluated by using 2, 2-Diphenyl-1-picrylhydrazyl (DPPH) method and by using different bacterial strains. These strains were *S. aureus* (Gram-positive bacteria) and *E. coli* (Gram-negative bacteria). The aim of the current study was to investigate the influence of cooking methods (boiling and microwave) on the antioxidant properties of different fruit and vegetable juices and to study the protection effect of vitamin C and natural lemon juice against ROS by using *E. coli* and *S. aureus*.

Materials and Methods

Chemical reagents:

2, 2-Diphenyl-1-picrylhydrazyl reagent was purchased from Sigma-Aldrich (Denmark); fully matured fresh fruits and vegetables were purchased from the local market. All other chemicals used were from Bio-Rad Laboratories and Sigma-Aldrich.

Vegetable and Fruit juices preparation:

Fully matured and high-quality vegetables and fruits, pepper, pomegranate, spinach, onion, orange, lemon, mandarin and tomato were used. Fresh fruits and vegetables were thoroughly washed, peeled, cut into small pieces (tomato, spinach and pepper were not peeled). The freshly squeezed juice was obtained by careful hand-squeezing of fruits and vegetables. The collected juices were filtered through 5-fold muslin cloth and stored in clean, tightly closed dark containers.

Cooking of fresh fruits and vegetables:

All fruits and vegetables were boiled for 30 minutes and when cooked by microwave, the cooking time was 15 minutes. The boiling was done on direct flame, while microwave power was adjusted at vegetable-cooking degree as directed by the microwave instructions.

Scavenging activity of fruit and vegetable juices by DPPH assay:

The radical scavenging ability of fruit and vegetable juices was tested on the basis of the radical scavenging effect on the DPPH free radical [19, 20]. The fruit and vegetable juices (25 µL to 200 µL/mL) were prepared in methanol. In clean and labeled test tubes, 4 mL of DPPH solution (0.002% in methanol) was mixed with 4 mL of different concentrations of fruit juices separately. The tubes were incubated under different experimental conditions and the optical density was measured at 517nm using spectrophotometer. The scavenging activity of the juices was calculated using the formula:

Scavenging activity (%) = [(A – B) / A] x 100, where A is absorbance of DPPH and B is absorbance of the mixture (DPPH and fruit juice) (14).

Preparation of bacterial suspension by using McFarland Latex Standard [21]:

E. coli (Gram-negative bacteria) and *S. aureus* (*Gram*-positive bacteria) were obtained from NCTC 8532 and ATCC BAA-1427 respectively. These strains were incubated in 5 ml of nutrient broth inoculated with bacterial cultures (<2 days old) at 37°C to make a standard bacterial suspension by using McFarland Latex Standard that comparable to a bacterial suspension of 1.5 X 10^8 CFU/ml. The media used in culturing bacterial broths was Müller-Hinton agar.

The effect of different concentration of hydrogen peroxide (H_2O_2) on bacterial growth:
Exactly 18-hr broth culture of the test bacteria isolates was suspended into different concentration of sterile hydrogen peroxide (H_2O_2). Two-fold serial dilution technique was used to measure the minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) [22]. A set of 9 tubes were prepared for each bacteria, 1000 µl of nutrient broth was added in all tubes, then 1000 µl of H_2O_2 solution (30 % v/v) was added in the first tube was mixed well. Then, from the solution of the first tube, 1000 µl were transferred to the second tube, mixed well. Then, 1000 µl of the solution in the second tube were transferred to the third, then from the third to the forth and so on, until the last tube, the 1000 µl were discarded. The extracts concentration in the first tube 15 % (v/v) and one-half dilution was carried out. Negative control tubes were prepared by using H_2O_2 solution. Positive control tubes were prepared by using bacterial solution only. The tubes were incubated at 37°C for 15 minutes. Finally, the suspension sub-cultured over the prepared Müller-Hinton agar plates and incubated at 37^0C for 24 hrs.

Bacterial growth after adding different concentration of H_2O_2 and lemon juice:
We added 100 µl of freshly squeezed lemon juice to the 500 µl of *E. coli* suspension (1.5 X 10^8 CFU/ml) that treated with H_2O_2 solution (its concentration were 3.75 and 1.87 (v/v) %) and incubated at 37°C for 1h. Then, *E. coli* was subcultured on MacConkey agar at 37 °C for 24 hr [23].

Results
The scavenging activity of different fruits and vegetables incubated under different conditions was measured. Different vegetable and some fruit juices showed different values of scavenging activity (Figure 1). Pepper showed the highest scavenging activity. Pomegranate, lemon and orange have convergent values, while, tomato, mandarin, spinach and onion exhibited low scavenging activity.

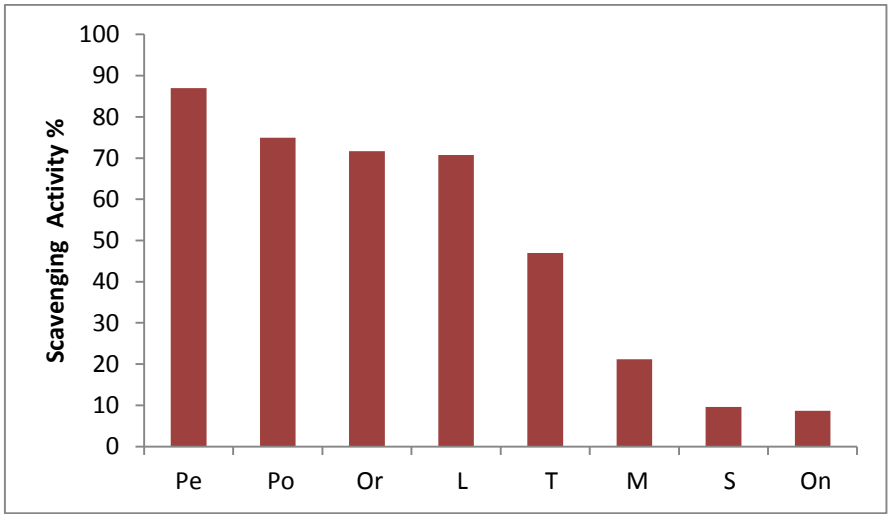

Figure 1: The vegetables and fruits used were pepper (Pe), spinach (S), onion (On), tomato (T), pomegranate (Po), orange (Or), lemon (L), and mandarin (M).

The effect of cooking method on the antioxidant activity of different vegetables and fruits was studied. Fresh fruit and vegetable juices were incubated in boiling water for 30 minutes. Lemon and orange showed a decrease in scavenging activity, while, pomegranate, tomato, spinach and onion exhibited an increase in scavenging activity (figure 2). Mandarin and pepper retain their antioxidant activity even after 30 minutes of boiling.
Fresh vegetable and fruit juices were incubated in microwave for 15 minutes and the scavenging activity was determined. It was found that the highest scavenging activity was shown by pomegranate, followed by pepper, orange, lemon, tomato, spinach, mandarin and onion (figure 3). On comparison microwave-cooked juices with fresh juice, we noticed that all vegetables and fruits juices showed an increase in scavenging activity except pepper.

The result of the susceptibility test of the bacterial strains to H_2O_2 showed that the MBC for *E. coli* was 3.75 % (v/v), while the MIC was 1.87 % (v/v). On the other hand, no H_2O_2 concentration could kill *S. aureus* even when the stock H_2O_2 solution (30% (v/v)) was used (Table 1).

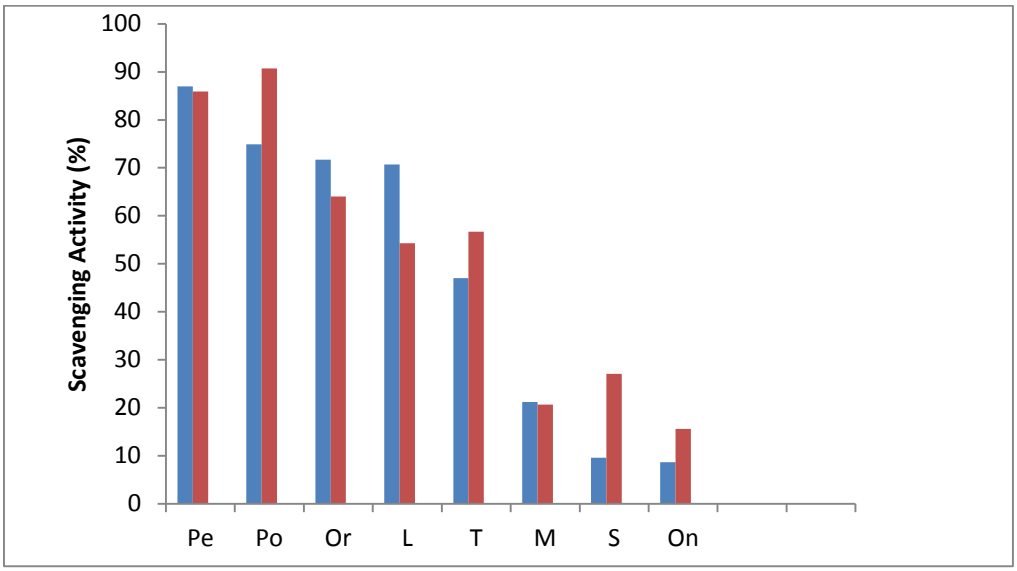

Figure 2: Scavenging activity of vegetable and fruit juices before (■) and after (■) 30 minutes of boiling

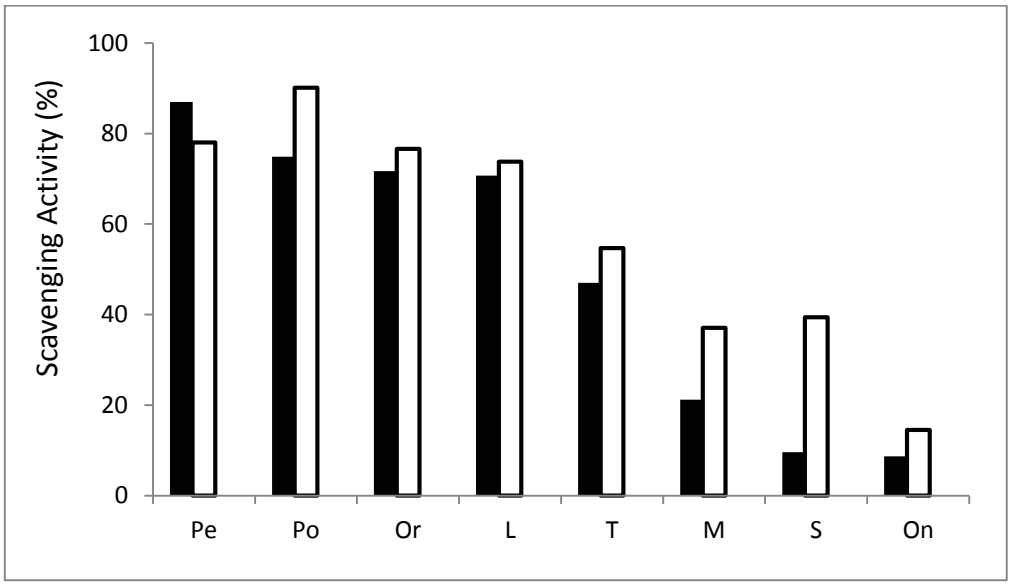

Figure 3: Scavenging activity of vegetable and fruit juices before (■) and after (□) 15 minutes of incubation in microwave. estimate and evaluate the antioxidant activities of natural sources of phytochemical compounds like fruits and vegetables

Discussion

The present study shows the scavenging activity and the antioxidants effect in different juices. The scavenging activity varies according to type of fruit and vegetabl. For example, orange juice has higher scavenging activity when compared to mandarin juice, while, pepper juice has higher scavenging activity when compared to onion juice, and this in agreement with previous studies [10]. The scavenging activity of different types of fresh juice depends on the content of fruit and vegetables. Most types of juices contain high rates of vitamins, mainly vitamin "C" and vitamin "A"[24]. In our study the scavenging activity was found to be highest in pepper juice followed by pomegranate, lemon, orange, tomato, mandarin, spinach and onion juices. This could be due to the presence of different compound with antioxidant capacity in different fruits and vegetables.

Table 1: bacterial treated with different concentration of hydrogen peroxide (positive control: bacterial suspension only; negative control: hydrogen peroxide only).

Hydrogen peroxide concentration (v/v) %	**Bacterial growth (*Escherichia coli)***	**Bacterial growth (*Staphylococcus aureus)***
30	No growth	+
15	No growth	++
7.5	No growth	+++
3.75	No growth	+++
1.87	+	+++
0.935	+	+++
0.467	++	+++
0.234	++	+++
0.117	++	+++
0.058	++	+++
Positive control	+++	+++
Negative control	No growth	No growth

E. coli growth was determined after adding 100 µl of freshly squeezed lemon juice to the 500 µl of *E. coli* suspension (1.5 X 10^8 CFU/ml) that treated with different H_2O_2 concentration. It was found that there is an increase in *E. coli* growth by increasing the concentration of fresh juice and the maximum growth was observed when undiluted juice was used (Table 2 and Table 3).

Table 2: bacterial treated with 3.75 hydrogen peroxide concentration (v/v) % and different concentration of lemon juice (positive control1: bacterial suspension only, positive control2: bacterial suspension with juice 100%, negative control hydrogen peroxide only).

Hydrogen peroxide concentration (v/v) %	**Lemon Juice concentration (v/v) %**	**Bacterial growth (*Escherichia coli)***
3.75	-	No growth
3.75	100	+++
3.75	50	++
3.75	25	+
Positive control		++++
Negative control		No growth
Bacteria with Vit. C (1g/3ml)		++++

Many studies have shown the effect of various cooking methods (boiling and microwave) on the scavenging activity of many fruits and vegetables [25-27]. The cooking methods may induce many changes in the content of phytochemicals found in fruits and vegetables. After 30 minutes of boiling of pepper, lemon and orange, the scavenging activity were lower than their uncooked counterparts. This is consistent with the suggestion of previous study [28]. The decrease in scavenging activity of cooked vegetables and fruits may be due to breakdown of different compound that may possess antioxidant activity [13, 25]. Other types of fruit and vegetable like pomegranate, tomato, spinach and onion showed an increase in scavenging activity after 30 minutes of boiling and this in agreement with another study [29]. The increase in antioxidant activity in cooked fruits and vegetables could be due to several reasons, liberation of a great amount of antioxidant components due to thermal destruction of vegetable cell walls, production of stronger scavenging antioxidants by thermal reaction, and suppression of the oxidation of antioxidants by thermal inactivation of oxidative enzymes [25].

Table 3: bacterial treated with 1.87 hydrogen peroxide concentration (v/v) % and different concentration of juice (positive control 1: bacterial suspension only, positive control 2: bacterial suspension with juice 100%, negative control hydrogen peroxide only).

Hydrogen peroxide concentration (v/v) %	**Lemon Juice concentration (v/v) %**	**Bacterial growth (*Escherichia coli)***
1.87	-	+
1.87	100	++++
1.87	50	+++
1.87	25	++
Positive control		++++
Negative control		No growth
Bacteria with Vit. C (1g/3ml)		++++

The scavenging activity was determined after the freshly fruit and vegetable juices were incubated in microwave for 15 minutes and it was found that the highest scavenging activity was shown by pomegranate, followed by pepper, orange, lemon, tomato, spinach, mandarin and onion. When antioxidant capacity of different fruit and vegetable juices were evaluated after being cooked in microwave, we noticed that all vegetable and fruit extracts showed an increase in scavenging activity except pepper. The increase in scavenging activity could be due to the activation and liberation of natural antioxidant compounds. On the other hand, another study showed that the amount of antioxidant activity decreased when different juices were cooked in microwave [26] and this was explained by the breakdown of antioxidant compouds during the heating.

The effect of H_2O_2 on bacterial growth was studied by using Gram-positive and Gram-negative bacteria, *S. aureus* (Gram-positive bacteria) resist the damaging effect by H_2O_2 because it is a catalase-positive bacteria [30]. When *E. coli* was used, it was found that 3.75% (v/v) H_2O_2 concentration was able to kill all suspended *E. coli*, but 1.87 % (v/v) H_2O_2 concentration kill some bacterial and few colonies were observed.

The protection effect of vitamin C and natural lemon juice against H_2O_2 was studied by using *E. coli* as a target. The MBC and MIC of H_2O_2 (3.75%, 1.87%) that kill or inhibit *E. coli* growth was used in the presence of different concentration of lemon juice or vitamin C. We noticed that the presence of lemon juice and vitamin C lead to increase *E. coli* growth as described in Table 2 and Table 3.

Conclusion

The scavenging activity varies for different fruit and vegetable. Boiling and microwaving were found to be good ways to retain the antioxidant capacity of some types of vegetable and fruit. The presence of lemon juice and vitamin C protect the bacteria from the damaging effect of H_2O_2 and promote their growth

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