Effect of boiling and microwave cooking on some antioxidant compounds in highly consumed vegetables in Egypt

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***Abstract*—**Effects of boiling and microwaving methods were studied on β-carotene, Vitamin C, total phenols, antioxidant capacity, and lycopene. Vegetable samples of artichoke, green haricot, okra, pea, squash, and tomato were used for the purpose of the study. There was a statistically significant reduction in β-carotene and Vitamin C when vegetables were boiled rather than micro-waved. Total phenolic content was significantly reduced ($p<0.05$) in vegetable samples. However, a slight increase in total phenolic content of green haricot was observed when boiled (23.28%), and micro-waved (16.72%). Boiling vegetable samples resulted in a decrease in total antioxidant capacity of green haricot, squash, and tomato. On the contrary, there was an increase in total antioxidant capacity of okra, pea, and artichoke. A similar observation was made when vegetable samples were microwaved. Both decrease and increase were not statistically significant except in artichoke. There was a significant difference in flavonoids concentration ($p<0.05$) between fresh vegetables and boiled vegetables. Boiled artichoke demonstrated larger antioxidants loss than microwaved artichoke. Boiling showed a statistically significant reduction of lycopene content in tomato, while microwaving showed a non-statistically significant reduction. It can be concluded that using a microwave oven in cooking vegetables plays an essential role in maintaining higher levels of nutritive values by retaining optimum levels of most antioxidants.

***Keywords*—** Vegetables, Microwave, Antioxidants.

I. Introduction

Eating wisely and saving food waste is a responsibility that maintains sustainability [1]. The World Health Organization (WHO) acknowledges that the global intake of vegetables is less than 20-50% of the recommended amount. In developed countries, the significantly low vegetable intake is due to the consumer's preferences for convenience foods and not the scarcity of the vegetables. Approximately 1.7 million (2.8%) of deaths worldwide are attributable to low vegetable consumption. Worldwide, insufficient intake of vegetables is estimated to cause around 14% of gastrointestinal cancer deaths, about 11% of ischemic heart disease deaths and about 9% of stroke deaths [2]. The consumption of fresh vegetables gives the consumer a variety of compounds that have a positive effect on human health. Due to the detection of many bioactive compounds in food with possible antioxidant activity, there has been an increased interest in the relationship between antioxidant and disease risks [3].

Vegetables are a good source of natural antioxidants such as carotenoids, vitamins, flavonoids, other phenolic compounds [4-7]. Epidemiological studies have shown a strong and consistent protective effect of vegetable consumption against the risk of several age-related diseases such as cancer, cardiovascular disease, cataract and macular degeneration [8-12]. To illustrate, regular consumption of tomatoes has been correlated with a reduced risk of various types of cancer [13-15] and heart diseases [16-17]. These positive effects are believed to be attributable to the high content of antioxidants, particularly lycopene [16]. Further, carotenoids are another type of antioxidants that have beneficial effects well-documented in the literature. In addition to their role as a precursor of vitamin A, carotenoids are valuable antioxidants, help in the prevention of atherosclerosis [18]. For example, β -Carotene is the well-known pro vitamin A, which can play a valuable role in promoting visual health. On the other hand, a wide

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range of non-vitamin A active carotenoids, have been shown to be primary components of the human macula pigment [19].

Nowadays, there is a global tendency to improve sustainable consumption patterns in both developed and developing countries through cooking devices such as microwave ovens. In Egypt, boiling is the most conventional method in cooking vegetables, whereas microwaving is a method that has been introduced only recently. These cooking methods are estimated to bring about a number of changes in physical characteristics and chemical composition of vegetables [12, 20], for example, [21] showed that boiling and baking had a small effect on the ascorbic acid, total phenolic, lycopene and antioxidant activity of the tomatoes. [12] indicate that cooking affected the antioxidant components and antioxidant activity of some vegetables. [22] found that thermal treatment decreased the total phenolic content in all vegetables, and antioxidant activity in some of them. [23] pointed out that processed vegetables show a wide range of phytochemical loss, and the technology in the food industry should be used to reduce the loss of antioxidants and micronutrients to the minimum by means of mild processes and the monitoring of each step of the transformation with due control assays. Therefore, the current study compares the effect of two cooking methods: boiling versus microwaving, on the antioxidants contents of vegetables; it aims at investigating best practices to maintain optimum nutritive value when boiling or microwaving vegetables. The study determines the concentrations of β carotene, vitamin C, total phenols, total antioxidant capacity, flavonoids, and lycopene in the following vegetables: artichoke, green haricot, okra, pea, squash, and tomato.

II. Materials and Methods

Sampling. Fresh vegetables are brought daily from villages to local markets in Alexandria in Egypt. Three kilograms of each fresh artichoke, green haricot, okra, pea, squash, and tomato were purchased in October 2013 at a local market in Alexandria, to be used as research materials. The samples were randomly selected off the shelf.

Preparation of Vegetable Samples. Vegetables were washed with tap water after removing inedible parts with a sharp knife. Vegetables dried on paper towel and were cut into small pieces or slices almost equal in size, then mixed well by hand to get a representative batch. Nine hundred grams were taken and divided into three portions (300 g for each application). One portion was retained raw; the other two portions were cooked using two different methods in triplicate, as explained below.

Boiling. One hundred and fifty ml of tap water was heated to reach the boiling point in a stainless steel pan. An amount of 100 grams of vegetable samples was added to the boiling water to be cooked for 5 minutes. The samples were drained off and cooled rapidly.

Microwave Cooking. An amount of 100 grams of vegetable samples was put in a glass dish. Six ml of tap water was added to the samples. The dish was covered with a cooking bag that had several holes. The samples were cooked for 5 minutes in a commercial Microwave Panasonic oven NN-S 2/5 WF, 800 watt/energy, 22L/capacity, 220 volt. The samples were drained off and cooled rapidly on ice.

Analytical Methods. Raw and cooked vegetables were homogenized in a blender (Moulinex–France) for 2 minutes for further analysis.

Determination of moisture content. It was carried out according to [24], the chemical analysis of fresh vegetables or just cooked was expressed on wet weight basis [25].

Determination of Vitamins and antioxidant content:

A. Determination of β carotene by HPLC

It was determined according to the method of [26]. For carotenoids extraction,0.5 g of

homogenized vegetable sample was extracted in 500 ml acetone: ethyl acetate (2:1, v/v)for 1 h in the dark. The extraction mixture was centrifuged at 10,000 rpm for 15 min. The organic phase was transferred to a new micro centrifuge tube and was evaporated under vacuum. The residues were dissolved in 100 ml ethyl acetate and 5 ml was injected on reverse phase HPLC. Analyses of carotenoids and ascorbic acid were performed using an Agilent 1200 series HPLC system (Santa Clara, CA), including a model G1311A quaternary pump, a model G1367Bauto sampler, a model G1316A column oven, and a model G13150 photodiode array detector. The column used was an Agilent ZORBAX Eclipse XDB-C18, 5 mm bead size, 4.6 mm_150 mm, connected with an Eclipse XDB-C18 guard column. The column temperature was controlled at 308°C during the HPLC runs. The flow rate was at 1 ml/ min. The mobile phases were acetonitrile:H2O: tri ethylamine (900:99:1,v/v/v) (A) and ethyl acetate (B). The gradient elution program was:0–5 min, 100–75% A; 5–10 min, 75–30% A ; 10–13 min, 30–0% A; 13–14 min,0–100% A; 14–15 min, 100% A. Data were collected at 440 nm, 477 nm and 296 nm. β-Carotene was identified based on the retention time and the spectrum as compared to the commercially available authentic standards.

B. Determination of Vitamin C by HPLC

Vitamin C was extracted according to the modified method of [27].The sample (10 g) was homogenized with an extracting solution containing meta-phosphoric acid (0.3M) and acetic acid (1.4 M). The mixture was placed in a conical flask (wrapped with aluminum foil) and agitated at 100 rpm with the aid of an orbital shaker for 15 min at room temperature. The mixture was then filtered through a Whatman No. 4 filter paper to obtain a clear extract. The ratio of the sample to extraction solution was 1 to 1. All samples were extracted in triplicates.

C. Determination of total phenolic and total antioxidant determination

The extraction procedure was a modification of the method described by [28]. Edible portion of each wet plant material was homogenized using blender immediately before extractions. A quantity (50 g) of each ground plant material was weighed separately, and 250 ml of50% aqueous ethanol (1:5 w/v) was added and mixed in vertical shaker for 6 h at 40°C inconstant temperature bath which did not vary more than two degrees either way. Then, the liquid extract was filtered and centrifuged at 2000 rpm for 20 min at room temperature to obtain a clear supernatant liquid which was used directly for DPPH and total phenolic compounds.

The antioxidant activity of the extracts, on the basis of the scavenging activity of The 2,2-Di (4-tert-octylphenyl)-1-picrylhydrazyl (DPPH) free radical, was determined by the method described by [29], 0.5 ml solution of DPPH radical in methanol was freshly prepared daily. An aliquot of 15-140 µl of each extract solution was mixed with 100 µl of methanolic DPPH radical to give final concentrations of 3- 28 mg extract/ml DPPH. The percent inhibition of radical scavenging ability was calculated as follow; % inhibition = [(Absorption of standard solution of ascorbic acid - Absorption of samples)/ Absorption of standard solution of ascorbic acid] *100

The amount of total phenolic contents in the extracts was determined calorimetrically with the Folin-Ciocalteu (FC) reagent, 20 µl of extract and 100 µl of undiluted (FC) reagent were added with incubation at 40°C in the dark for 30 min for colour development was performed and then reading absorbance at 765 nm by UV-1650PC visible spectrophotometer. The absorbance obtained was converted to gallic acid equivalent as milligrams per gram (GAE/g), using gallic acid standard curve.

D. Determination of Flavonoids (hesperidin) by HPLC

Hydrolysis, extraction, and recovery test according to [30], where100 mg sample placed in a 20 ml tube containing 10 mg ascorbic acid dissolved in 5 ml of acidified methanol (1.2 M HCl)

was flushed with N2 air for 30 sec and then refluxed at 80°C for 2hour. After cooled down to room temperature, the sample was centrifuged at 4000g for 10 min. Supernatant, approximately 2 ml was taken and filtered through 0.2 µm syringe filter (Millipore, Bedford, MA). The filtrate was kept at 10°C for HPLC analyses within 12 h. Flavonoids (hesperidin) were separated using the HPLC system equipped with a Water 2695 separation module and an Agilent Zorbax ODS column (3.5µm, 4.6 x 150 mm) at 35 °C using a gradient from0 −15 min, 1 to 25% acetonitrile (ACN) in 1% aqueous formic acid (FA); and 15−50 min, 25%−40% ACN in 1% aqueous FA at a flow rate of 0.7 ml/min. The column elute was monitored using a Waters 2996 photo diode array detector (250−700 nm). Identification and quantification of individual flavonoids was carried out using commercial standards.

E. Determination of lycopene

Lycopene concentration of tomato was measured using a HPLC system described by [21, 31].

Statistical analysis. All data were recorded as means ± SE and Duncan comparisons were carried out to test any significant differences between raw and cooked vegetables. The quantitative data presented for each treated vegetable was based on the average of three replicates and expressed on a wet weight basis (n= 3).

Chemicals. DPPH and Folin–Ciacalteu reagents were purchased from Sigma Aldrich Egypt, Other chemicals used were all analytical grade and bought from "Al Gomhoreya" company-Egypt.

III. Results

Results in Tables I-VI represent the concentrations of β -Carotene, ascorbic acid, total phenolic compounds, lycopene and antioxidant activity of the cooked vegetables compared to raw.

TABLE I:
EFFECT OF BOILING AND MICROWAVE COOKING PRACTICES ON B CAROTENE CONTENT (MG/100G)

Vegetable	Fresh Mean ± SD	Boiled Mean ± SD	% Change	Microwave Mean ± SD	% Change
Artichoke	1.37 ± 0.19	0.27 ± 0.03	19.59	1.26 ± 0.14	8.04
Green haricot	1.42 ± 0.16	0.43 ± 0.05**	69.50	1.27 ± 0.14	11.10
Okra	1.49 ± 0.17	0.87 ± 0.10**	42.22	1.30 ± 0.14	13.14
Pea	2.24 ± 0.25	0.86 ± 0.10**	61.72	1.61 ± 0.18	28.16
Squash	2.14 ± 0.24	0.62 ± 0.07**	71.02	1.53 ± 0.17	28.60
Tomato	5.96 ± 0.66	1.29 ± 0.14**	78.22	3.20 ± 0.24	46.32

Data are expressed as means ± SE of triplicate experiments (on wet basis)*Significant at $p < 0.05$. **Significant $p < 0.01$

TABLE II.
EFFECT OF BOILING AND MICROWAVE COOKING PRACTICES ON VITAMIN C CONTENT (MG/100G)

Vegetable	Fresh Mean ± SD	Boiled Mean ± SD	% Change	Microwave Mean ± SD	% Change
Artichoke	7.40 ± 0.82	5.20 ± 0.69	29.72	6.20 ± 0.58	16.21
Green haricot	7.90 ± 0.88	1.79 ± 0.20*	77.34	5.60 ± 0.62	29.11
Okra	6.10 ± 0.68	3.50 ± 0.39*	42.62	5.40 ± 0.60	11.47
Pea	3.79 ± 0.42	2.10 ± 0.23	44.59	3.30 ± 0.37	12.92
Squash	11.20 ± 1.24	1.60 ± 0.18**	85.71	10.6 ± 1.18	5.35
Tomato	12.79 ± 1.42	3.50 ± 0.39*	72.63	7.45 ± 0.02	41.75

Data are expressed as means ± SE of triplicate experiments (on wet basis)*Significant at $p<0.05$.**Significant $p<0.01$

TABLE III.
EFFECT OF BOILING AND MICROWAVE COOKING PRACTICES ON TOTAL PHENOLIC CONTENT OF DIFFERENT VEGETABLES (MG/100G)

Vegetable	Fresh Mean ± SD	Boiled Mean ± SD	% Change	Microwave Mean ± SD	% Change
Artichoke	0.11 ± 0.21	0.054± 0.01*	49.05	0.09 ± 0.02	15.09
Green haricot	0.34 ± 0.03	0.413 ±0.18	+23.28	0.39 ± 0.02	+16.72
Okra	0.84 ± 0.26	0.02 ± 0.01**	72.599	0.53± 0.05*	37.57
Pea	0.11 ± 0.33	0.05 ± 0.01*	52.22	0.09 ±0.02	20.37
Squash	0.09 ± 0.36	0.04 ± 0.09**	63.13	0.08 ±0.02	24.24
Tomato	2.18 ± 0.31	1.41 ± 0.12*	35.32	1.82 ± 0.01	16.51

Data are expressed as means ± SE of triplicate experiments (on wet basis)*Significant at $p<0.05$. **Significant $p<0.01$

TABLE IV.
EFFECT OF BOILING AND MICROWAVE COOKING PRACTICES ON TOTAL ANTIOXIDANT CAPACITY OF VEGETABLES

Vegetable	Fresh Mean ± SD	Boiled Mean ± SD	% Change	Microwave Mean ± SD	% Change
Artichoke	46.74 + 0.03	68.60 + 0.02*	+ 46.77	69.53 + 0.01*	+48.76
Green haricot	63.02 + 0.03	60.69 + 0.02	3.70	44.69 +0.01	29.01
Okra	65.58 + 0.02	67.81 + 0.02	+3.40	85.11 + 0.01*	+29.78
Pea	47.67 +0.03	51.02 + 0.02	+7.03	43.58 + 0.04	8.58
Squash	71.16 + 0.02	53.72 + 0.01	24.51	42.25 + 0.02*	40.63
Tomato	57.40 + 0.02	66.50 + 0.02	15.85	59.23 + 0.02	+3.19

Data are expressed as means ± SE of triplicate experiments (on wet basis)*Significant at p<0.05.

TABLE V.
HESPERIDIN'S CONTENT OF VEGETABLES AT BOILING AND MICROWAVE COOKING (MG/100G)

Vegetable	Fresh Mean ± SD	Boiled Mean ± SD	% Change	Microwave Mean ± SD	% Change
Artichoke	94.00 ± 10.44	2.05 ± 0.13**	97.81	24.60 ± 2.73*	73.82
Green haricot	2.73 ± 0.30	0.96 ± 0.02*	64.83	1.86 ± 0.62	31.86
Okra	76.00 ± 8.44	2.04 ± 0.23**	74.31	24.08 ± 2.68*	68.31
Pea	78.61 ± 8.73	5.10 ± 0.57**	93.51	69.38 ± 0.68*	11.74
Squash	5.18 ± 0.58	1.17 ± 0.13*	77.41	2.60 ± 0.18	49.80
Tomato	10.19 ±1.13	6.57 ±0.42*	35.52	8.87 ±10.67	12.95

Data are expressed as means ± SE of triplicate experiments (on wet basis)*Significant at p<0.05. **Significant p<0.01

TABLE VI .
LYCOPENE CONTENT IN TOMATO AT DIFFERENT COOKING TREATMENTS (MG/100G)

Vegetable	Fresh Mean ± SD	Boiled Mean ± SD	% Change	Microwave Mean ± SD	% Change
Tomato	7.80 ± 0.34	3.20 ± 0.36*	59.03	5.90 ± 0.83	24.35

Data are expressed as means ± SE of triplicate experiments (on wet basis)*Significant at p<0.05

IV. DISCUSSION

A. Effect of boiling and microwave cooking practices on β carotene

Table I compares the concentration of β-carotene in fresh vegetables and its concentration in both boiled and micro-waved vegetables. The highest loss of β -carotene (80%) was detected when artichoke was boiled. In contrast, when microwaving artichoke, only 8.04% of B-carotene was lost. There was a significant difference in β -carotene concentration ($p<0.05$) between fresh vegetables and boiled vegetables. On the other hand, no statistically significant difference was detected in β -carotene between fresh vegetables and micro-waved vegetables. Both boiling and microwaving caused a substantial loss of β-carotene, which could be explained by the consequent leaching of molecules into water and their instability at the boiling temperatures of the boiling process (100° C). Some researchers have reported similar results of β carotene loss from vegetables, including spinach, amaranth and fenugreek, during cooking procedures, such as boiling, stewing, frying, blanching and pressure cooking [32, 33]. In contrast, [25] found no lutein content in the water after boiling vegetables in it, suggesting that no carotenoids leached out water during the boiling of the vegetables.

B. Effect of boiling and microwave cooking practices on ascorbic acid (vitamin C) content

Table II compares the concentration of ascorbic acid (Vitamin C) in fresh vegetables and its concentration in both boiled and micro-waved vegetables. To start with, the highest loss of Vitamin C (85.71%) was detected when squash was boiled causing highly statistical significance ($p<0.001$). In contrast, when microwaving squash, only 5.35% of Vitamin C was lost. There were other significant differences in Vitamin C concentrations between fresh and boiled green haricot, okra, and tomato ($p<0.05$). Artichoke and pea showed no significant difference in Vitamin C between fresh and boiled samples. No statistically significant difference was detected in Vitamin C between fresh vegetables and micro-waved vegetables. This indicates that cooking affects the retention of ascorbic acid in the tissues. [34] pointed out that the cooking procedures could result in significant losses of vitamin [32, 33] reported losses

of ascorbic acid from vegetables including spinach and fenugreek, during cooking procedures, such as boiling, stewing, frying, blanching, and pressure cooking.

C. Effect of boiling and microwave cooking practices on total phenolic acid

Table III compares the concentration of total phenols in fresh vegetables and its concentration in both boiled and micro-waved vegetables. Total phenolic content was significantly reduced ($p<0.05$) in vegetable samples. However, a slight increase in total phenolic content of green haricot was observed when boiled (23.28%), and micro-waved (16.72%). This was not statistically significant. Research data on total phenols in cooked vegetables is very limited. [12] reported that raw broccoli floret retained (28.1%) and (28.4%) of total phenolic content when boiled and micro-waved respectively. [35] reported that boiling broccoli for 15 minutes retained 18% of total phenols. Remaining total phenols leached into cooking water. [36] found that after blanching, total phenols decreased or increased depending on the type of vegetables, a result which is in consistency with the current study.

D. Effect of boiling and microwaving on the total antioxidant capacity (mg/g GAE)

Table IV compares the concentration of total antioxidant capacity (mg/g GAE) in fresh vegetables and its concentration in both boiled and micro-waved vegetables. Boiling vegetable sample resulted in a decrease in total antioxidant capacity of green haricot, squash, and tomato. On the contrary, there was an increase in total antioxidant capacity of okra, pea, and artichoke. Both decrease and increase were not statistically significant except in artichoke. To illustrate, boiling and microwaving artichoke caused the highest significant increase in total antioxidant capacity (46.77%, 48.76% respectively). The study by [12] showed that there was no significant difference in total antioxidant capacity between boiling and microwaving. Peas had a lower total antioxidant capacity than spinach [22], which conforms to the results of the present study. [12] reported that raw broccoli florets had 60.5% of total antioxidant capacity as measured by DPPH when boiled and micro-waved; the florets retained 35% and 34.7% of total antioxidant capacity respectively. However, results of the current study showed that total antioxidant capacity of raw vegetables was less than that reported by [12]. [22] reported that total antioxidant capacity of vegetables boiled for one minute was similar to the capacity of fresh ones.

E. Effect of boiling and microwave cooking practices on Flavonoids (hesperidins) content

Table V compares the concentration of flavonoids in fresh vegetables and their concentration in both boiled and micro-waved vegetables. The highest loss of flavonoids (97.81%) was detected when artichoke was boiled. Similarly, when microwaving artichoke, 73.82% of flavonoids were lost. There was a significant difference in flavonoids concentration ($p<0.05$) between fresh vegetables and boiled vegetables. On the other hand, no statistically significant difference was detected in flavonoids between fresh vegetables and micro-waved vegetables except in artichoke (73.82%) and pea (68.31%). Both boiling and microwaving caused a substantial loss of flavonoids, which could be explained by the consequent leaching of molecules into water and their instability at the boiling temperatures of the boiling process (100° C).

Flavonoids are potent antioxidant compounds found in plants that have been found, for instance, to inhibit tumour development [37]. Flavonoids also have a wide range of other potential benefits [38, 39].

F. Effect of boiling and microwaving on lycopene content in tomato

Table VI illustrates the concentration of lycopene in fresh tomato and its concentration in both boiled and micro-waved tomato. Boiling tomato decreased lycopene content by 59.03%,

which was statistically significant. Likewise, when tomato was micro-waved, lycopene content was reduced by 24.35% showing no statistical significance. Similar studies reported that lycopene in tomato was dramatically dropped during microwave heating and baking [40, 41].

V. Conclusion

To begin with, there was a statistically significant reduction in β-carotene and Vitamin C when vegetables were boiled, whereas there was no significant difference when micro-waved. Consequently, it can be concluded that micro-waved vegetables retain higher amount of β-carotene and Vitamin C than boiled vegetables. Further, total phenolic content was significantly reduced in vegetable samples. However, a slight increase in total phenolic content was detected in both boiled and micro-waved green haricot, which was not statistically significant. Moreover, boiling and microwaving almost had the same effect on total antioxidant capacity. However, levels of total antioxidant capacity vary according to the type of vegetable. In addition, both boiling and microwaving caused a substantial loss of flavonoids. This loss was statistically significant in boiling, whereas insignificant in microwaving vegetables. Likewise, boiling showed a statistically significant reduction of lycopene content in tomato, while microwaving showed a non-statistically significant reduction. It can be concluded that using a microwave oven in cooking vegetables plays an essential role in maintaining higher levels of nutritive values by retaining optimum levels of most antioxidants.

VI. Recommendations

It is highly recommended to use microwave devices to help towards better retention of antioxidants in vegetables. Moreover, as there is a loss of antioxidant compounds in boiled vegetables, shorter cooking time guarantees better antioxidant retention. Furthermore, the person in charge of preparing food is advised to consider food nutritional quality by choosing cooking methods that avoid the loss of antioxidants. In addition, in this study, it is estimated that using household devices (microwave oven) could save natural antioxidants and avoid depending on antioxidant supplementation. Nevertheless, the results of this study should be used in organizing workshops and seminars to enhance health education. This should take place on a narrow scope represented in family, as well as a broader scope including catering in hotels, restaurants, hospitals, etc. Last but not least, further studies are needed to investigate the effect of cooking times and conventional Egyptian cooking methods on the nutritional quality of food.

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