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EFFECT OF COOKING ON ASCORBIC ACID AND TOTAL POLYPHENOL CONTENT AND ANTIOXIDANT ACTIVITY OF SELECTED VEGETABLES

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ABSTRACT

The study was carried out to determine the effect of cooking on ascorbic acid content (AAC), total polyphenol content (TPC) and antioxidant activity (AA) of selected vegetables: beetroot, cabbage, eggplant, green bean, onion and spinach obtained from local market of Sri Lanka were subjected for cooking for 7 minutes at 78±2°C. AAC and TPC of water extracted vegetables were determined using 2, 6-dichlorophenol-indophenol titrimetric method and Folin-Ciocalteu method, respectively. AA of ethanol extracted vegetables was measured using DPPH radical scavenging assay and expressed as IC_{50} values. AAC varied widely among the raw vegetables from 42.40 mg ml^{-1} to 7.07 mg ml^{-1} and AAC in all selected vegetables were reduced by 60-80% with the effect of cooking. TPC of beetroot, cabbage and eggplant showed increase with cooking by 12.28%, 16.54%, 19.48% respectively. AA of eggplant was increased after cooking by 22.08% with the liberation of high amounts of antioxidant components due to the thermal destruction of cell walls. Other selected vegetables were reduced their AA under the same conditions. Beetroot, cabbage, eggplant, green bean, onion and spinach were showed IC_{50} values as 0.128, 0.161, 0.048, 0.055, 0.950, 0.072 in mg ml^{-1} respectively & cooking showed both effect.

Keywords: Antioxidant activity, Ascorbic acid content, Cooking, Total polyphenol content, Vegetables

INTRODUCTION

Vegetables and other plant food as fruits, beans, nuts and whole grains are recommended for human diet (Loliger, 1991). As a result of unhealthy food habits of people, non communicable diseases (NCD) such as diabetes, cancer, obesity, cardiovascular diseases are spreading at an alarming rate. Researchers found that oxidative damages cause these NCD due to free radical generation. Free radicals contribute to more than one hundred disorders in humans (Nisha, 2009). Proper antioxidant source is the only way of deactivating free radicals which are generating within human body. On the basis of nutrient composition in vegetables able to generate natural antioxidant sources as enzymes, tocopherol, carotenoids, ascorbic acid, polyphenols etc. Harmful effect of free radicals which results degenerative diseases like diabetes, cancer, atherosclerosis, hypertension etc. Can be prevented.

Substantial evidence indicates that foods containing antioxidants may be of major importance in prevention of ncds. There are, however, growing consensus among scientists that a combination of antioxidants, rather than single entities, may be more effective over the long term consumption. The increased intake of natural antioxidants, particularly the antioxidative compounds present in vegetables contribute to the antioxidant capacity of plasma and these constituents are reported to mitigate the damage caused by the oxidative stress (Lin & Yang, 2007)

Common meal of Sri Lankan diet consists of rice and curry together with vegetables and are taken in cooked form as curries. According to the socioeconomic survey, vegetable consumption is higher than that of fruit consumption in Sri Lanka. Beans, eggplant, cabbage, pumpkin, carrot and beet root are the most frequently consumed vegetables. Among the leafy vegetables, Mukunuwanna (*Alternanthera sessilis*), Gotukola (*Centella asiatica*) and Spinach (*Spinacia oleracea*) are the most consumed (Consumer Finances and Socioeconomic Survey, 2003/04). Cooking and processing may affect the antioxidant content in vegetables, especially components such as ascorbic acid, tocopherols, carotenoids and polyphenols (Abdelrahmann *et al.*, 2014). Objective of this study was to identify the effect of cooking on AAC, TPC and AA of few selected vegetables to customize cooking method to improve total antioxidant activity will become great impact to improve human health.

MATERIALS AND METHODS

Raw vegetables

Beetroot (*Beta vulgaris*), cabbage (*Brassica oleracea*), eggplant (S*olanum melongina*), green bean (*Phaseolus vulgaris*), onion (*Allium cepa*) and spinach (*Spinacia oleracea*) samples which has been harvested at correct stage of maturity and fresh condition were obtained from the local market, Kandy.

Sample preparation

The vegetables were cooked in the laboratory, after cleaning and washing with water (in consumer conditions) and after separating the non edible portion and cut into small pieces, placed 50g into vessel and added 100 ml water. Two treatments were practiced as raw and cooked. Average household level cooking condition was applied such as all vegetables samples were cooked with water at 78 °C for 7 minutes. Dini and others (2008) mentioned that 78 °C is the best cooking time which were previously established for each vegetable by an informal testing panel consisting of 3 trained panellists, so that vegetables had the colour and texture of home-cooked products. Referring to cooking time of vegetables 7 to 10 minutes time period consider as domestic cooking time (Chuah *et al*., 2009). According to that referred literature 7 minutes time was selected as common cooking time for selected vegetables.

Determination of ascorbic acid content (AAC)

The 2,6-Dichlorophenol Indophenol titrimetric method (Rangana, 1977) was used to determine the ascorbic acid content. Two grams of Sample was blend with 20 ml of 3% HPO_3, then filtered and centrifuged. Five millilitres aliquot of extract was titrated with standard dye to a pink end point which should persist at least 15 seconds.

$$\text{Ascorbic acid per 100 g or ml} = \frac{\text{Titer} \times \text{Dye factor} \times \text{Volume made up} \times 100}{\begin{array}{c}\text{Aliquot of extract} \\ \text{Taken for estimation}\end{array} \times \begin{array}{c}\text{Weight of the sample} \\ \text{taken for the estimation}\end{array}}$$

Determination of the total polyphenol content (TPC)

The amount of total polyphenols was determined according to the method of Abdelrahmann *et al*. (2014). 20µl of raw and heat treated sample extracts were mixed with 100 µl of Folin-Ciocalteu's reagent, left for 3 minutes and subsequently 300 µl of sodium carbonate (0.7 M) was added and vortexed. The absorbance of the resulting mixture was measured at 725 nm using a UV visible spectrophotometer after leaving for 30 minutes at the room temperature (25 °C). The results were expressed as mg gallic acid equivalents (GAE) per litre of extract using a gallic acid (50 – 500 mg/l) standard curve.

Determination of DPPH radical scavenging capacity

Antioxidant capacity of the 80% methanol extracts of raw and cooked vegetables were assessed using 2,2-diphenyl-1- picrylhydrazylhydrate (DPPH) radical scavenging activity (Abdelrahmann *et al*., 2014) with minor modifications. For DPPH radical scavenging assay, five different concentrations ranging between 0.1 and 1.0 mg/ml of each extract (0.5 ml) was mixed with 2.5 ml of methanolic DPPH radical (0.1 mM). After leaving for 20 minutes in the dark at room temperature, the absorbance was recorded at 517 nm using a UV visible spectrophotometer. The radical scavenging activity (RSA) was calculated as percentage DPPH discoloration using the following equation.

$$RSA\ (\%)=[(A_{DPPH}-A_{Sample})/A_{DPPH}]\times 100$$

Where, A_{Sample} is the absorbance of the solution containing the extract after 20 minutes and A_{DPPH} is the absorbance of the DPPH solution devoid of extract. Results were expressed as IC_{50} value that denotes the concentration of the sample required to scavenge 50% of DPPH radicals.

Analysis of data

Five replicates were conducted for each analysis and vegetable. Chemically analyzed data were subjected to one-way Analysis of Variance (ANOVA) and the significant of differences between means were assessed using Complete Randomized Designing (CRD) performed by Minitab version 17 Statistical Software.

RESULTS AND DISCUSSION

Ascorbic acid content

Ascorbic acid which present in vegetables and its antioxidant capacity is associated with reduction of cancer incidence (Choi *et al*., 2006). Considering the results (Table 1), cooking decreases the AAC of all the selected vegetables at different rates. The highest reduction (80%) of AAC was observed in onion after 7 min cooking by 80%.

Table 1. Ascorbic Acid Content mg/100 g of selected vegetables when raw and 7 min. and reduction % after 7 min. cooking.

Selected vegetables	Non- cooked (mg/100g)	Cooked for 7 min (mg/100g)	AAC reduction After 7 min. (%)
Beetroot	$7.07^{a}\pm0.00$	$1.77^{b}\pm0.00$	74.96
Cabbage	$17.67^{a}\pm0.00$	$7.07^{b}\pm0.00$	59.98
Eggplant	$14.13^{a}\pm0.00$	$3.53^{b}\pm0.00$	75.01
Green bean	$10.60^{a}\pm0.00$	$3.53^{b}\pm0.00$	66.69
Onion	$35.33^{a}\pm0.00$	$7.07^{b}\pm0.00$	79.98
Spinach	$42.40^{a}\pm0.00$	$10.60^{b}\pm0.00$	75.00

Note: Values are Means ± Standard error of 5 replicates. Treatment means having common letter(s) are not significantly different by CRD 5% in each raw.

Previous studies have shown that cooking reduces AAC in fruits and vegetables (Choi *et al.*, 2006). High heat can reduce the vitamin C or the AAC of vegetables, and when heat and water are combined, as they are in cooking, can see significant reduction of vitamin C. Vitamin C is one of the least stable of all vitamins and is oxidized readily by light, air and when heated (Choi *et al.*, 2006). This means that heating in water causes the vitamin to leach out of the food into the water and also to be oxidized, first to dehydroascorbic acid and then to diketogluonic acid (Choi *et al.*, 2006). This last compound has no ascorbic acid activity at all and is irreversible.

Ascorbic acid is involved in detoxification of toxic substances. Also as an antioxidant that reacts with histamine and peroxide for reducing inflammatory symptoms. AAC in the vegetables shows positive relationship with antioxidant activity of them, thus, reduction of the AAC from the cooking may directly affect the AA of the vegetables. In order to gain more health benefits from the ascorbic acid to the human body people should avoid high heat treatments like cooking with water. Also people should consume fresh vegetables and should consume vegetables with more AAC contain vegetables such as spinach.

Total polyphenol content

Phenolic constituents are one of the major groups of compounds serving as primary antioxidants, especially as free radical terminators (Marja *et al.,* 1999). Table 2 shows the Total phenolic content in raw form which varied from 305.772 to 018.690 in mg GAE/100g.

Table 2. Total Polyphenol Content of water extracts of vegetable extracts before and after cooking.

Selected vegetables	Non- cooked (mg GAE/100g)	Cooked for 7 min (mg GAE/100g)	TPC reduction After 7 min. (%)
Beetroot	$305.77^{b}\pm0.06$	$343.32^{a}\pm0.16$	-12.28
Cabbage	$43.21^{b}\pm0.00$	$51.63^{a}\pm0.03$	-19.48
Eggplant	$53.47^{a}\pm0.24$	$62.31^{b}\pm0.00$	-16.54
Green bean	$18.69^{a}\pm0.00$	$3.53^{b}\pm0.00$	66.69
Onion	$67.23^{a}\pm0.00$	$28.42^{b}\pm0.03$	57.72
Spinach	$126.30^{a}\pm0.00$	$95.21^{b}\pm0.00$	24.61

Note: Values are Means ± Standard error of 5 replicates. Treatment means having common letter(s) are not significantly different by CRD 5% in each raw.

While TPC of eggplant, cabbage and beetroot were increased with cooking, the values were reduced in all other vegetables. All selected vegetables were shown significant difference in raw and cooked treatments (Table 2). Broccoli, sweet potato, carrot also able to increase TPC with cooking as shown Lin and Yang (2007). There is other ample evidence indicating that positive cooking effect on TPC of vegetables. These authors suggest that, liberation of phenolic compounds were enhanced by cooking in specific vegetables. Among selected vegetables Eggplant, Cabbage and Beetroot have that specific feature (Table 2).

Considering the phenolic compounds in the Eggplants, which had the third highest value in the selected six vegetable has a significant antioxidant activity than other vegetables due to its important phenolic compounds. Generally, the peel contains higher amounts of phenolics, anthocyanins, and flavonols than pulp tissue of egg plant (Barberán *et al.,* 2004). Compared to pulp, brinjal peel contains higher amounts of those compounds. Especially, the anthocyanin content in the peel is much higher than the pulp (Lin & Yang, 2007). It is very important to note that cooking increases TPC levels in some vegetables due to the disruption of cell walls, which liberate soluble phenolic compounds from insoluble ester bonds (Lin & Yang, 2007).

In Chaga mushroom (*Inonotus obliquus*) TPC was significantly increased after cooking. These results could explain that after cooking bound phenolic compounds converts to free forms and released into cytosol as phenolic acids (Choi *et al.,* 2006). These authors suggest that, liberation of phenolic compounds were enhanced by cooking. Applying the same principle, conclusions can be made for the increment of TPC in Eggplant, Cabbage and Beetroot after cooking.

DPPH radical scavenging activity and total antioxidant capacity

The DPPH radical scavenging activity of the tested extracts under non-cook (raw) and cook treatments are summarized in Table 3. The DPPH radical scavenging capacity was expressed as IC_{50} value which is inversely proportional to the antioxidant activity (Trantis *et al*., 2005).

The IC_{50} value of non-cooked (raw) 80% methanol extracts of vegetables ranged between 0.950 mg/ml to 0.048 mg/ml with Eggplant showing the highest antioxidant activity followed by the other five vegetables; Green bean, Spinach, Beetroot, Cabbage, and Onion, respectively. As TPC, cooking shows both positive and negative effect to the AA. Eggplant shows specific features and increase AA by cooking while all other selected vegetables were decreased their AA. Four possibilities are suggested for the increase in antioxidant activity of some vegetables after cooking (Gorinstein *et al.,* 2005): (1) the liberation of high amounts of antioxidant components due to the thermal destruction of cell walls and sub cellular compartments; (2) the production of stronger radical scavenging antioxidants by thermal chemical reaction; and/or (3) suppression of the oxidation capacity of antioxidants by thermal inactivation of oxidative enzymes; (4) production of new nonnutrient antioxidants or the formation of novel compounds such as Maillard (Trantis *et al.,* 2005). However, it is not clear to what extent each possible factor contributes to the increase in activity (Abdelrahmann *et al*., 2014).). It has recently been shown that the thermal processing of sweet corn, tomato, and other vegetables increases antioxidant activity (Abdelrahmann *et al*., 2014).).

Vegetables showing an IC_{50} value of less than 1 mg/ ml is categorized as vegetables with extremely high antioxidant potential (Trantis *et al.,* 2005) According to the IC_{50} value, all selected (six) vegetables can be categorized as vegetables with high antioxidant potential.

Table 3. The DPPH radical scavenging capacity of selected vegetables before and after cooking

Selected vegetables	Non- cooked IC_{50} value (mg/ml)	Cooked for 7 min IC_{50} value (mg/ml)	AA reduction After 7 min. (%)
Beetroot	$0.13^{b}±0.00$	$0.20^{a}±0.00$	-58.59
Cabbage	$0.16^{b}±0.00$	$0.39^{a}±0.00$	-140.37
Eggplant	$0.05^{a}±0.00$	$0.04^{b}±0.00$	22.08
Green bean	$0.06^{b}±0.00$	$0.09^{a}±0.00$	-67.27
Onion	$0.95^{b}±0.00$	$1.39^{a}±0.00$	-46.86
Spinach	$0.07^{b}±0.00$	$0.09^{a}±0.00$	-26.38

Note: Values are Means ± Standard error of 5 replicates. Treatment means having common letter(s) are not significantly different by CRD 5% in each raw.

When vegetables are subjected to cooking processes, such as boiling, pressure-cooking, microwaving, baking, griddling, deep frying, variations appear in their antioxidant activity or radical scavenging capacity. These variations depend on the vegetables themselves (bioactive structures), the cooking method, the bioavailability of phenolics (Das *et al*., 1997), temperature, the localization of the structures in the vegetables, cutting, chopping, stability of the structure to heat (Das *et al*., 1997)., the synergic activity of the structures, and on the reaction systems assayed (for example, β-carotene is an efficient singlet oxygen quencher but is not a hydrogen (Abdelrahmann *et al*., 2014).

In the case of boiling or pressure-cooking occurs lixiviation phenomenon that leads to a 64% loss of total carotenoids and 49% loss of total phenolics (Das *et al*., 1997). Phenols enter the cooking water and complex phenol proteins are found, reducing drastically by 90% or more according to Cao *et al.* (1996). The concentration of phenolic acids is highest in the outer layers of some vegetables (Das *et al*., 1997) and these are extremely exposed to water reducing antioxidant power of some vegetables such as pea, spinach, cauliflower, and cabbage (Chuah *et al*., 2008).

However, although total phenolics are usually stored in vegetables in pectin or cellulose networks and can be released during thermal processing. Individual phenolics may sometimes increase because heat can break supra molecular structures, releasing the phenolic sugar glicosidic bounds, which react better with the Folin–Ciocalteau reagent (Chuah *et al*., 2008). On the other hand, boiling may decrease the activity by decreasing ascorbic acid (Abdelrahmann *et al*., 2014), higher activity may also occur as consequence of the inactivation of oxidative enzymes such as ascorbate oxidase (Abdelrahmann *et al*., 2014). This fact reduces the browning potential and, although chlorogenic acid decreases, the ascorbic acid allows, its concentration to be retained for a longer time (Amarowicz *et al.*, 2000) On the other hand, boiling retains the active components in the cooked tissue (Abdelrahmann *et al*., 2014). Results of this study for the most of the vegetables also agree with this. With the reduction of AAC and TPC most of the situation vegetables showed reduction in AA. Cooking method should alter with considering all factors to gain maximum benefits from the daily consuming vegetables in Sri Lanka.

Cooking temperature also affects the antioxidant potential (Noda *et al*., 2000) of the vegetables. More Researches using different cooking temperatures will be needed to find proper cooking temperatures that can be used in domestic levels. Each vegetable has its own way of antioxidant activity during cooking. It is important to have a proper data base for daily consuming vegetables and their proper cooking methods if we are to utilize the natural nutrient profile of these vegetables. After identifying vegetables with rich antioxidant profiles, they can be used to develop pharmaceuticals instead of chemically developed pharmaceuticals for improve human health against the most of the non-communicable diseases.

CONCLUSIONS

The selected vegetables can be categorized as vegetables with high antioxidant potential. Effect of cooking on AAC, TPC and AA of selected vegetables shows both positive and negative behaviour. AAC in the all studied vegetables were drastically reduced with the effect of cooking. TPC of beetroot, cabbage and eggplant were increased with cooking and it was decreased in rest of the vegetables tested. Eggplant was the only vegetable which showed increased levels of AA with cooking. AA was reduced in rest of the vegetables during cooking.

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