

## Effect of Storage and Cooking Time on the Antioxidant Properties of Two Commonly Used Ghanaian Vegetables

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**Abstract:** Green leafy vegetables are good sources of antioxidants. This study was designed to evaluate the effect of storage and cooking time on the antioxidant properties of two commonly used Ghanaian vegetables: *Corchorus olitorius* and *Solanum macrocarpon* locally known as “adeimey” and “gboma” respectively. The storage conditions were: Left to stand overnight at room temperature, refrigerated for one day and refrigerated for one week, and cooked for ten and thirty minutes at 100°C. The reducing power and the total carotenoid content were determined by measuring absorbance using a Shimadzu UV-120-02 spectrophotometer at 700nm and 450 nm respectively. The free radical scavenging activity was determined by the 1, 1-diphenyl-2-picryl-hydrazil (DPPH) method. The antioxidant activity (AOA) ranged from 91.59 to 38.42%. The total carotenoid content (TCC) varied from 5.10 to 27.30 mg/100 g. The reducing power ranged from 9.52 to 50.59%. The results show that *Solanum macrocarpon* and *Corchorus olitorius* are good sources of antioxidants with the later exhibiting higher antioxidant properties than the former. However the AOA, TCC and reducing power decreased drastically when the vegetables were refrigerated for one day and one week and cooked for thirty minutes at 100°C. Similarly the AOA, TCC and reducing power decreased slightly when the stored vegetables were cooked for ten minutes. These findings suggest modification in the storage and cooking practices of these green leafy vegetables to ensure retention of antioxidants for the best nutritional value and health benefit. The results also indicate that in areas where refrigeration is not possible, vegetables can be left overnight at room temperature to preserve the antioxidants in them.

**Key words:** Antioxidant, cooking and storage practices, leafy vegetables, nutritional value

### INTRODUCTION

The importance of leafy vegetables in nutrition and health cannot be overemphasized. According to Lu and Foo (2000), a diet rich in vegetables is recommended along with fruits and whole grains since such a diet has an inverse association with the risk of chronic diseases. In recent times the medicinal value of plant source foods has assumed a more important dimension owing to the discovery that their extracts contain not only micronutrients but also a diverse array of secondary metabolites with antioxidant potential.

Vegetables are known to contain antioxidants necessary in neutralizing free radicals which are known human chemical hazards. Free radicals are generated under a number of conditions such as drinking alcohol, smoking and exercise (Akinmoladun *et al.*, 2007). They are, however, often produced in normal cellular metabolism due to oxidation of biomolecules for the production of energy to fuel biological processes.

However, the uncontrolled production of oxygen-derived free radicals is involved in the onset of many diseases such as cancer, rheumatoid arthritis, cirrhosis and arteriosclerosis as well as in degenerative processes associated with ageing (Akinmoladun *et al.*, 2007).

Humans are protected against free radical damage by oxidative enzymes such as Superoxide Dismutase (SOD) and Catalase (CAT), or chemical compounds mostly found in plant foods such as  $\alpha$ -tocopherol, ascorbic acid, carotenoids, polyphenols and glutathione (Niki *et al.*, 1994). Many plants have been identified as good sources of natural antioxidants such as tocopherols, vitamin C, carotenoids and polyphenols which are responsible for maintaining good health and protect against coronary heart diseases and cancer (Arabshahi *et al.*, 2007; Castenmiller *et al.*, 2002; Javanmardi *et al.*, 2003; Kaur and Kapoor, 2001).

Hence, free radicals accumulate when the mechanism of antioxidant protection becomes unbalanced. However, available evidence indicates that reparative processes do

not fully eliminate free radical-induced damage of biological macromolecules (Maulick and Das, 1995). Thus, a more effective way is the prevention of oxidant-induced damage by reducing the levels of reactive chemical species with unpaired electrons (free radicals) to the barest minimum and reinforcing natural antioxidant action (Maulick and Das, 1995). For this reason natural antioxidant-containing foods, such as vegetables and spices, are being extensively studied for their capacity to protect cells from damage brought on by oxidative stress.

The Ghanaian local market offers a variety of vegetables as food resources but little work has been done to study the antioxidant properties in these vegetables in order to understand their fullest nutritional and health benefits. It is against this background that this study was conducted to evaluate the effect of storage and cooking time on the antioxidant properties of *Corchorus olitorius* (adeimey) and *Solanum macrocarpon* (gboma) with the aim of determining the changes in concentration and suggesting the best storage and cooking time that maintains or loses minimal amount of antioxidant properties.

## MATERIALS AND METHODS

Two green leafy vegetables namely *Corchorus olitorius* and *Solanum macrocarpon* locally called "adeimey" and "gboma" respectively, were purchased from Madina market (a local market) in Accra-Ghana early in the morning.

**Preparation of extracts:** The edible portions of the vegetables were plucked, weighed and subjected to different storage conditions: fresh, left overnight at room temperature, refrigerated for one day and refrigerated for one week at 4°C. These were then subjected to different cooking times: 0, 10 and 30 min. Each of the treated vegetables (0.5000 g) was ground separately in a mortar with 25 mL of methanol. The mixture was filtered using Whatman No. 4 filter paper and the residue was re-extracted under the same conditions until the extraction solvents became colorless. The extract obtained was filtered using Whatman No. 4 filter paper and the filtrate was collected into a newly labeled falcon tube. The total volume of the filtrate was recorded and then stored at 4°C to prevent oxidative damage.

**Determination of antioxidant activity, reducing power and total carotenoid content:** The reducing power of the methanol extract of each vegetable was determined according to the method of Oyaizu (1986) with slight modifications. The free radical scavenging activity of each vegetable was measured by the 1, 1-diphenyl-2-

picryl-hydrazil (DPPH) method as described by Blois (2002). The total carotenoid content was determined by measuring the absorbance of the extract at 450 nm using a Shimadzu UV-120-02 spectrophotometer.

**Statistical analysis:** The data were represented as mean $\pm$ standard deviation of three experimental determinations. Between sample and within sample variations were evaluated by using one-way analysis of variance and Duncan test to show where the differences are significant.  $p<0.05$  were regarded as significant. Graphical presentations of the data were done in excel and single factor ANOVA was done using Statgraphics (version 3.1).

## RESULTS AND DISCUSSION

Antioxidants are compounds that can delay or prevent the oxidation of lipids or other molecules by inhibiting the initiation or propagation of an oxidizing chain reaction and by many other mechanisms and thus prevent diseases (Zheng and Wang, 2001). The antioxidant activity (AOA), expressed as percent inhibition of oxidation, ranged from as high as  $91.59\pm0.00\%$  to as low as  $38.42\pm0.01\%$ . The Total Carotenoid Content (TCC) ranged from as high as  $27.30\pm0.00$  mg/100g to as low as  $5.10\pm0.00$  mg/100g. The Reducing Power (RP) ranged from  $50.59\pm0.02\%$  to  $9.52\pm0.00\%$  as shown in Table 1. Cooking these vegetables for both ten and thirty minutes decreased their AOA, RP and TCC with the latter recording the least value for each of the storage conditions and cooking times as shown in Table 1.

The results show that *Solanum macrocarpon* and *Corchorus olitorius* are good sources of antioxidants with the later exhibiting higher antioxidant properties than the former. This is because there is enough evidence that there exist a direct correlation between the concentration of antioxidant compounds and the antioxidant activity/property (Lan, 2007; Lakenbrink *et al.*, 2000; Lu and Foo, 2000).

The results also revealed variations in the Antioxidant Activity (AOA) of the vegetables ( $p<0.001$  for control and extract). The free radical scavenging activities of *Solanum macrocarpon* and *Corchorus olitorius* were evaluated based on their ability to trap the DPPH radical. At 517 nm in the visible spectrum, the DPPH free radical showed a strong absorption band (deep purple color) and as the odd electrons became paired in the presence of the antioxidant, this strong absorption band began to disappear and the resulting discoloration was found to stoichiometrically correspond with the number of electrons taken up. The DPPH scavenging effect (%) is normally used as a measure of the

Table 1: Total carotenoid content, antioxidant activity and reducing power of *Solanum macrocarpon* and *Corchorus olitorius*

Treatment	<i>Solanum macrocarpon</i>			<i>Corchorus olitorius</i>		
	Total carotenoids (mg/100 g)	Antioxidant activity (%)	Reducing power (%)	Total carotenoids (mg/100 g)	Antioxidant activity (%)	Reducing power (%)
Fresh, uncooked	16.80±0.01 <sup>14</sup>	89.87±0.00 <sup>g,h,k</sup>	47.62±0.01 <sup>L,M</sup>	17.00±0.00 <sup>14</sup>	90.73±0.00 <sup>h,k</sup>	36.42±0.00 <sup>G,H</sup>
Left overnight, uncooked	18.00±0.00 <sup>13</sup>	90.84±0.00 <sup>h,k</sup>	44.20±0.02 <sup>J,L</sup>	27.30±0.00 <sup>15</sup>	91.59±0.00 <sup>h,k</sup>	10.53±0.00 <sup>A</sup>
Refrigerated for one day, uncooked	14.90±0.00 <sup>12</sup>	88.59±0.00 <sup>g,h,k</sup>	47.68±0.01 <sup>I,J</sup>	16.80±0.01 <sup>14</sup>	89.63±0.00 <sup>g,h,k</sup>	38.81±0.01 <sup>I</sup>
Refrigerated for one week, uncooked	12.10±0.00 <sup>9</sup>	86.07±0.00 <sup>g,h</sup>	50.59±0.02 <sup>P</sup>	16.10±0.00 <sup>13</sup>	87.31±0.00 <sup>g,h</sup>	42.69±0.01 <sup>J</sup>
Fresh, cooked for 10 min	8.30±0.01 <sup>6</sup>	86.60±0.02 <sup>g,h</sup>	41.32±0.01 <sup>J</sup>	13.40±0.01 <sup>10</sup>	87.79±0.01 <sup>g,h</sup>	18.40±0.01 <sup>C</sup>
Left overnight, cooked for 10 min	13.20±0.01 <sup>10</sup>	86.58±0.00 <sup>g,h</sup>	27.76±0.01 <sup>D</sup>	15.00±0.00 <sup>12</sup>	85.11±0.00 <sup>g,h</sup>	10.12±0.00 <sup>A</sup>
Refrigerated for one day, cooked for 10 min	7.60±0.01 <sup>5</sup>	71.62±0.01 <sup>C,D,E</sup>	45.87±0.01 <sup>I,J</sup>	10.80±0.00 <sup>8</sup>	87.08±0.01 <sup>g,h</sup>	30.24±0.01 <sup>E</sup>
Refrigerated for one week, cooked for 10 min	7.10±0.00 <sup>4</sup>	68.53±0.01 <sup>C</sup>	47.09±0.00 <sup>L,M</sup>	9.00±0.00 <sup>7</sup>	77.15±0.02 <sup>E,F</sup>	38.55±0.01 <sup>H,I</sup>
Fresh, cooked for 30 min	6.60±0.00 <sup>3</sup>	82.66±0.03 <sup>D,E</sup>	27.18±0.03 <sup>D</sup>	10.40±0.01 <sup>8</sup>	83.06±0.01 <sup>F,G</sup>	12.36±0.06 <sup>B</sup>
Left overnight, cooked for 30 min	10.60±0.00 <sup>8</sup>	83.34±0.01 <sup>F,G</sup>	15.51±0.02 <sup>B</sup>	14.50±0.00 <sup>11</sup>	84.69±0.01 <sup>g,h</sup>	9.52±0.00 <sup>A</sup>
Refrigerated for one day, cooked for 30 min	5.70±0.01 <sup>2</sup>	67.04±0.01 <sup>C</sup>	33.88±0.01 <sup>F</sup>	7.40±0.01 <sup>4,5</sup>	70.65±0.01 <sup>C,D</sup>	17.56±0.01 <sup>C</sup>
Refrigerated for one week, cooked for 30 min	5.10±0.00 <sup>1</sup>	38.42±0.01 <sup>A</sup>	36.10±0.01 <sup>F,G</sup>	7.10±0.00 <sup>4</sup>	59.87±0.05 <sup>B</sup>	19.00±0.00 <sup>C</sup>
Standard	95.00±0.00 <sup>k</sup>	8.14±0.01 <sup>A</sup>		95.00±0.00 <sup>k</sup>	95.00±0.00 <sup>k</sup>	8.1416±0.01 <sup>A</sup>

1-14, a-k and A-P within a column with the same superscript means that there are no significant differences but those with different superscript show significant differences

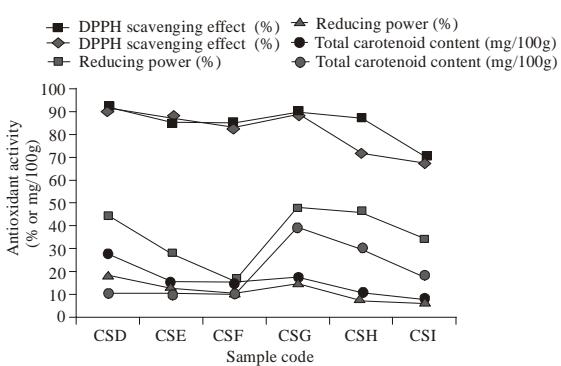


Fig. 1: Antioxidant activity of vegetables

Left overnight	CSD
Left overnight, cooked for 10 min	CSE
Left overnight, cooked for 30 min	CSF
Refrigerated for one day	CSG
Refrigerated for one day, cooked for 10 min	CSH
Refrigerated for one day, cooked for 30 min	CSI

antioxidant activity of a substance (Blois, 2002). Hence the DPPH scavenging effect (%) or the antioxidant activity was compared to 10 mM Butylated Hydroxytoluene (BHT) used as a standard since the actual concentration of the active antioxidants were not known. Butylated Hydroxytoluene (BHT) is one of the major antioxidants used in foods (Fennema, 1985).

In comparison to the standard, the storage condition as well as cooking decreased the antioxidant activity (Fig. 1). The extent of destruction was more pronounced in those vegetables that were cooked for 30 min (Fig. 1). It can, therefore, be said that prolonging the cooking time destroyed most of the heat labile antioxidant compounds in the vegetables. The results of this present study is consistent with the findings of Elizalde-González and Hernández-Ogarcía (2007) who reported that the concentration of antioxidant compounds in fresh samples or uncooked samples were higher than the cooked ones. This confirms why the antioxidant activity of the uncooked vegetables was higher than the cooked ones.

It has been well documented that different storage conditions affect the nutrient composition of foods. This is further confirmed by the results of this study since increasing the storage time decreased significantly the antioxidant activity of the vegetables (Table 1). The results from this present work show that vegetables left overnight had a better scavenging capacity than that obtained freshly from the market. This could probably be due to the significant differences in their moisture content. Nevertheless, it is advisable that in areas where there is no electricity, such vegetables could be stored for not more than one day. It is important to note that the vegetables studied were left overnight at room temperature.

There was also a statistically significant difference between the mean AOA, RP and TCC from one sample to another at the 95% confidence level ( $p<0.05$ ) as shown in

Table 2: Correlation between total carotenoid content, antioxidant activity and reducing power of *Corchorus olitorius* and *Solanum macrocarpon*

Variables	<i>Corchorus olitorius</i>	<i>Solanum macrocarpon</i>
Antioxidant activity and total carotenoid content	0.701**	0.725**
Total carotenoid content and reducing power	-0.117	0.286
Antioxidant activity and reducing power	-0.012	0.083

\*\*: Correlation is significant at the 0.01 level (1-tailed)

Table 2. Antioxidant activity [DPPH scavenging effect (%)] correlated significantly and positively with the total carotenoid content ( $r = 0.701, 0.725$ ;  $p < 0.05$ ) respectively in both vegetables. However, there was no linear correlation between antioxidant activity and reducing power ( $r = -0.012, 0.083$ ;  $p > 0.05$ ) respectively; neither was there a correlation between reducing power and total carotenoid content respectively ( $r = -0.117, 0.286$ ) as presented in Table 2. That is, the AOA correlated significantly and positively with TCC. This suggests that TCC of a vegetable is a good predictor of the antioxidant activity of the same vegetable. Thus, the TCC provides a source of dietary antioxidants in addition to imparting flavor to the food. Furthermore, the absence of a linear correlation between the DPPH scavenging effect and Reducing Power (RP) suggests that reducing power does not fully characterize the antioxidant activity. This confirms earlier reports by Odukoya *et al.* (2005) who reported that there was no linear correlation between total antioxidant activity and reducing power ( $r = -0.53$ ) among 20 extracts from 12 Nigerian dietary spices.

Again, the antioxidant activity [DPPH scavenging effect (%)] was very high as compared to the reducing power and the total carotenoid content. However, the different storage conditions as well as cooking times significantly decreased the antioxidant activity as shown in Fig. 1. Antioxidant activity represents the capability of scavenging free radicals and offering hydrogen atoms; higher antioxidant activity therefore indicates stronger capability of scavenging free radicals (Blois, 2002). The presence of different antioxidant substances occurring in plant tissues, especially fruits and vegetables, makes it relatively difficult to measure each antioxidant component separately. This therefore limited our ability to measure the antioxidant activity of each of the antioxidant substances present in the studied vegetables. Several methods, including oxygen radical absorption capacity method, ferric reducing antioxidant capacity method, liposome assay, lipid peroxidation and total oxyradical scavenging capacity assay, have therefore been developed in recent years to calculate the total antioxidant activity of biological samples (Zheng and Wang, 2001) as we did in this present study.

## CONCLUSION

The green leafy vegetables, *Solanum macrocarpon* and *Corchorus olitorius* are good sources of antioxidants with variations in their Antioxidant Activity (AOA). *Corchorus olitorius* exhibited more antioxidant potential than *Solanum macrocarpon*. Storage as well as cooking times reduced the AOA and most of the heat labile antioxidant compounds (carotenoids level) in the vegetables. It is very important to reduce heat treatment and storage time to ensure best nutritional value of these vegetables. Most importantly, in areas where refrigeration is not available, vegetables can be left overnight at room temperature to preserve the antioxidants in them.

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