

## Carotenoids content of *Corchorus olitorius* and *Solanum macrocarpon* - commonly used Ghanaian vegetables

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### Abstract

Green leafy vegetables are rich in carotenoids. The study investigated the effect of duration of storage and cooking time on the carotenoids in *Corchorus olitorius* and *Solanum macrocarpon*, locally known as 'adeimey' and 'gboma' respectively. The storage conditions were: overnight at room temperature (25°C), refrigeration for one day and a week, and cooking at 100°C for thirty minutes. The three individual carotenoids and total carotenoid content (TCC) were quantified using HPLC and by measuring absorbance at 450 nm, respectively. The TCC varied from 17.1 mg/100 g to 670.2 mg/100 g. The  $\beta$ -carotene content ranged from 7.5 mg/100 g to 196.3 mg/100 g while lutein ranged from 1.4 mg/100 g to 112.8 mg/100 g. The zeaxanthin content ranged from 0.7 mg/100 g to 16.3 mg/100 g. These results show that the two green leafy vegetables are good sources of carotenoids. There were variations in the concentrations of carotenoids in *Solanum macrocarpon* and *Corchorus olitorius* with the latter having a higher carotenoids content than the former.  $\beta$ -carotene was the most predominant carotenoid whilst zeaxanthin was the lowest in the vegetables studied. It was observed that the  $\beta$ -carotene, lutein, zeaxanthin and the total carotenoid contents decreased drastically when the vegetables were left to stand overnight at room temperature, refrigerated for one day and a week and then cooked for thirty minutes. These findings suggest modification in the storage and cooking practices of these green leafy vegetables to ensure retention of the carotenoids for the best nutritional value and health benefit; hence the need for policy towards nutrition education on vegetable processing and storage.

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### Introduction

Even though scientific evidence is clear that everyone needs to make more food choices from vegetables, majority of people do not eat at all or eat enough vegetables. Consumption of quality and different types of vegetables are skewed towards higher socio-economic groupings, because of cost and social class associated with some vegetables especially in developing countries. Vegetables are rich sources of phytochemicals especially carotenoids. Carotenoids are nature's most widespread pigments. They are particularly important for their provitamin A and antioxidant roles. Carotenoids are useful for their role in photosynthesis and photoprotection in plant tissues. The photoprotective role of carotenoids is attributed to their ability to stop and inactivate reactive oxygen species (ROS) formed from exposure of light and air (Sharma *et al.*, 2012; Chandra *et al.*, 2012). This photoprotection role has been linked to its antioxidant activity in human health. However, recent evidence suggests that the body's total

endogenous antioxidant protection mechanism seems unresponsive to high doses of dietary antioxidants (Halliwell, 2012).

In humans, therefore, the unique and important biological function of carotenoids is their role in vision, growth, cell differentiation, and other physiological processes (Arigony *et al.*, 2013). Of the several carotenoids included in the diet of humans,  $\beta$ -carotene is the most common, followed by  $\alpha$ -carotene,  $\beta$ -cryptoxanthin, lutein, zeaxanthin and lycopene (Chandra *et al.*, 2012).  $\beta$ -carotene,  $\alpha$ -carotene and  $\beta$ -cryptoxanthin are able to function as vitamin A precursors. Zeaxanthin and lutein are excellent lipid-soluble carotenoids which unlike  $\beta$ -carotene, do not convert into vitamin A (retinol) in the body but their main function is to maintain normal vision of the human eye macula. Within the central macula, zeaxanthin is the predominant component, whereas in the peripheral retina lutein predominates (Le and Xiao-Ming, 2010). They protect the underlying tissues of the eye from photo-toxic damage by filtering of blue light.

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Provitamin A carotenoids are a major source of retinol in Ghana and other developing countries. In Ghana, Togo, Sierra Leone and Cote d'Ivoire, young leaves of *Corchorus olitorius* and *Solanum macrocarpon* are used to produce a popular mucilaginous dish as a staple. The young leaves of these vegetables exhibit antioxidant potentials *in vitro* (Steiner-Asiedu *et al.*, 2012) but work has not been done to quantify the carotenoids in order to understand their full nutritional and health benefits. This study therefore quantified the carotenoids in *Corchorus olitorius* and *Solanum macrocarpon*, locally known respectively as 'adeimey' and 'gboma' that were stored under different conditions. The findings are to form the basis for nutrition education for the general populace who usually do not consume fresh raw vegetables but cooked or stored over time which has implications on the nutritional quality.

## Materials and Methods

### Sample collection

Two green leafy vegetables (500 g each) namely *Corchorus olitorius* (adeimey) and *Solanum macrocarpon* (gboma) were purchased fresh early in the morning from three different women in the open market in Accra-Ghana. Convenience sampling was used to obtain the samples. The collected samples were labelled accordingly and transported to the laboratory.

### Preparation of extracts

The edible portions of the vegetables from the three different retailers were plucked and thoroughly mixed, weighed and given different storage conditions as usually practiced in homes: fresh, left overnight, refrigerated for one day and refrigerated for one week. About 0.15 g and 0.50 g of the leafy vegetables were further subjected respectively to cooking for zero minute and thirty minutes in 50ml of deionized water, while the uncooked samples (0 min) served as control. Each quantity of the treated vegetables together with the stock was ground separately with 1.13 g of pyrogallol with a mortar and pestle in 25 ml of cold acetone. Pyrogallol was added before extraction to prevent the oxidation of the active compounds. The mixture was filtered and the residue was re-extracted under the same conditions until the extraction solvents became colourless and the filtrates were pulled together. The filtrate was partitioned on 20 ml petroleum ether in a 500 ml separating funnel. The acetone was washed away with about 1000 ml of distilled water by draining away the aqueous layer. The petroleum ether layer was dried by passing it

through anhydrous sodium sulphate on a cotton wool at the base of a funnel. The extract was collected into a newly labelled falcon tube. The total volume of the extract was recorded and then stored at 4°C to prevent oxidative damage (Nanasombat *et al.*, 2012).

### Determination of moisture content

Two previously dried and cooled moisture dishes were weighed with their covers. Approximately 2.0 g of finely cut-up edible portions of vegetables were weighed into the previously dried, cooled and weighed moisture dishes. The samples were dried at 105°C overnight for 6-8 hours using the air-oven (cover off inside oven). After air drying, the samples were cooled in a desiccator, weighed again and the percentage moisture determined.

### Determination of total carotenoid content

The total carotenoid content (TCC) of the petroleum ether extract of each vegetable was determined by measuring the absorbance of the extract at 450 nm using a Shimadzu UV-120-02 spectrophotometer. The TCC of each extract was calculated based on the measured absorbance, conversion factor and moisture content (de Carvalho *et al.*, 2012).

### Quantification of $\beta$ -carotene, Lutein and Zeaxanthin

The  $\beta$ -carotene, Lutein and Zeaxanthin content of the petroleum ether extract of each vegetable was quantified by using HPLC as described by Elizalde-González and Hernández-Ogarcía (2007) with slight modifications. In an effort to model theoretically the retention of the carotenoids under study, two different mobile phases on a normal phase Zorbax ODS 4.6 mm ID\*25 cm column with petroleum ether as eluent was used. The mobile phases were methyl alcohol/ acetonitrile/ethyl acetate 2:6:2 (v/v) and hexane/dichloromethane 6:1 (v/v) at a flow rate of 0.8 mL/min and UV detection at 450 nm. The latter mobile phase was used to elute  $\beta$ -carotene while the former was used to elute lutein and zeaxanthin.

Briefly, petroleum ether extract of each vegetable (1 ml) was taken and evaporated to dryness under a steam of nitrogen gas. It was then reconstituted with a known volume of the mobile phase. The reconstituted extract was vortex for thirty seconds and then 20  $\mu$ l injected onto an HPLC column and their corresponding areas were obtained. Typical reference chromatograms from a  $\beta$ -carotene, lutein and zeaxanthin references respectively and those from the extracts were compared. The concentration of  $\beta$ -carotene, lutein and zeaxanthin in the extracts were calculated based on their corresponding areas

obtained, the concentration of the standards and their moisture content.

### Statistical analysis

The data were presented as mean of two independent experimental determinations and each was replicated once. The experiment was repeated for two independent determinations provided the difference is greater than 0.5. Single factor ANOVA was done using Statgraphics (version 3.1) and  $p < 0.05$  was regarded significant. Graphical presentations of the data were done in excel.

## Results and Discussion

### Total carotenoid content, $\beta$ -carotene, lutein and zeaxanthin

Several techniques can be used to quantify carotenoids in vegetables, including spectrophotometry for simple colorimetric estimations, and HPLC for isolation and quantification of individual carotenoids as described by Rodriguez-Amaya and Kimura (2004). A saponification pre-treatment of samples may be used for better peak separation in HPLC (Rodriguez-Amaya and Kimura, 2004) but the vegetables under study were not subjected to saponification because they are poor sources of esterified carotenoids. The mobile phase, methyl alcohol/acetonitrile/ethyl acetate 2:6:2 (v/v) was used to elute lutein and zeaxanthin whilst hexane/dichloromethane 6:1 (v/v) was used to elute  $\beta$ -carotene. The latter mobile phase was used because it was suspected that the presence of peroxides in the chromatography mobile phase retained  $\beta$ -carotene. The presence of peroxides in the chromatography mobile phase could probably be due to the ethyl acetate used in the former mobile phase (E-Siong and Swan-Choo, 2001).

The total carotenoid content (TCC) varied from 670.2 mg/100 g to 17.1 mg/100 g. The  $\beta$ -carotene content ranged from as high as 196.3 mg/100 g to as low as 7.5 mg/100 g. The lutein content also ranged from as high as 112.8 mg/100 g to as low as 1.4 mg/100 g. The zeaxanthin content ranged from 0.7 mg/100 g to 16.3 mg/100 g (Table 1). The  $\beta$ -carotene was the most predominant of the carotenoids present in the vegetables whilst zeaxanthin was the lowest. Cooking for thirty minutes decreased drastically the  $\beta$ -carotene, zeaxanthin, lutein and the total carotenoid contents of the vegetables after storage in a refrigerator for one week (Fig. 1). Similarly, it has been reported that the concentrations of  $\beta$ -carotene and lutein were higher in the fresh form of four tomato species compared with those obtained from

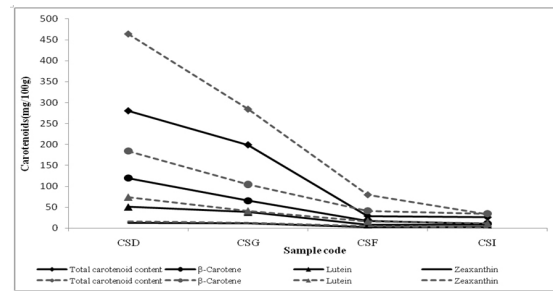


Figure 1. Carotenoids in vegetables

Keys: Full line represents carotenoids in *Solanum macrocarpon*, Dashed line represents carotenoids in *Corchorus olerarius*. Left overnight, CSD; Refrigerated for one day, CSG; Left overnight, cooked for 30 minutes, CSF; and Refrigerated for one day, cooked for 30 minutes, CSI.

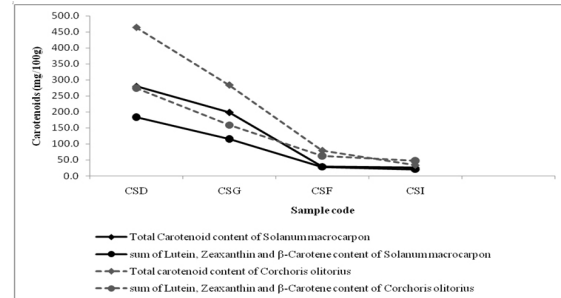


Figure 2. Comparison of Total Carotenoid Content with sum of Lutein, Zeaxanthin and  $\beta$ - carotene content of the vegetables

Keys: Left overnight, CSD; Refrigerated for one day, CSG; Left overnight, cooked for 30 minutes, CSF; and Refrigerated for one day, cooked for 30 minutes, CSI.

the tomato species subjected to pressure cooking (Elizalde-González and Hernández-Ogarcía, 2007). This presupposes that during cooking, the electron-rich carotenoid molecules underwent oxidation in both *Solanum macrocarpon* and *Corchorus olerarius* and therefore were lost to retention. Loss of  $\beta$ -carotene was greatest in both vegetables probably because it has the most number of conjugated double bonds which easily underwent oxidation (Rodriguez-Amaya and Kimura, 2004). Elizalde-González and Hernández-Ogarcía (2007) therefore suggested that, thermal processes such as microwaving are milder in the oxidation of the double bonds in the carotenoids under investigation. Storage over time decreased drastically  $\beta$ -carotene, zeaxanthin, lutein and the total carotenoid contents of the vegetables (Fig.1). This may be due to enzymatic catalyzed oxidation of the carotenoids in the vegetables on exposure to oxygen (Rodriguez-Amaya and Kimura, 2004).

Comparing our chromatograms to those obtained by Elizalde-González and Hernández-Ogarcía (2007) indicated that the leaves contain other carotenoids that were not quantified. The presence of these unidentified peaks may indicate that the vegetables could be good sources of other carotenoids hence their consumption should be encouraged. It was expected that, the  $\beta$ -carotene, lutein and zeaxanthin contents should sum up to the TCC. This is because  $\beta$ -carotene, lutein and zeaxanthin are among the

Table 1. Effect of storage and cooking time on the Carotenoid content in *Solanum macrocarpon* and *Corchorus olitorius* (mg/100 g)

Treatment	<i>Solanum macrocarpon</i>				<i>Corchorus olitorius</i>			
	Total carotenoids	Beta carotene	Lutein	Zeaxanthin	Total carotenoids	Beta carotene	Lutein	Zeaxanthin
Fresh, uncooked	291.26±0.08	140.05±0.13	90.38 ±0.08	15.31 ±0.04	670.18±0.05	196.32±0.08	112.75±0.05	16.32 ±0.16
Left overnight, uncooked	279.94±0.04	119.78±0.16	50.83 ±0.05	13.27 ±0.11	463.93±0.05	184.21 ±0.21	74.44 ±0.11	15.91 ±0.06
Refrigerated for one day, uncooked	198.81±0.03	65.52 ±0.34	38.33 ±0.13	11.98 ±0.01	284.14 ±0.46	104.45±0.04	41.47 ±0.05	12.95 ±0.04
Refrigerated for one week, uncooked	97.21 ±0.03	50.36 ±0.06	22.43 ±0.11	4.82 ±0.06	242.00±0.12	55.54 ±0.12	34.78 ±0.09	11.20 ±0.15
Fresh, cooked for 30 minutes	41.02 ±0.07	23.90 ±0.07	12.02 ±0.01	3.20 ±0.01	68.60 ±0.06	40.99 ±0.30	20.89 ±0.16	4.91 ±0.07
Left overnight, cooked for 30 minutes	29.18 ±0.11	17.70 ±0.06	8.44 ±0.09	2.29 ±0.03	79.53 ±0.11	41.67 ±0.08	16.71 ±0.02	4.60 ±0.09
Refrigerated for one day, cooked for 30 minutes	26.10 ±0.04	10.59 ±0.33	8.05 ±0.08	2.22 ±0.04	33.70 ±0.06	34.78 ±0.17	11.53 ±0.10	1.49 ±0.06
Refrigerated for one week, cooked for 30 minutes	17.05 ±0.37	7.47 ±0.08	1.39 ±0.06	0.68 ±0.15	21.47 ±0.04	9.11 ±0.04	4.57 ±0.13	1.44 ±0.09

most abundant carotenoids in nature (Chandra *et al.*, 2012). Statistical analysis as shown in Fig. 2 indicates that there was significant difference between the summation of  $\beta$ -carotene, lutein and zeaxanthin and the TCC ( $p < 0.001$ ). This could be due to the fact that, other carotenoids not quantified due to the absence of standards may also have contributed significantly to the TCC.

#### Recommended dietary allowance for $\beta$ -carotene, lutein and zeaxanthin

The recommended dietary allowance (RDA) is the average daily dietary intake level sufficient to meet the nutrient requirement of nearly (97-98%) healthy individuals in each age and gender group (Gibson, 2005). There is no RDA for  $\beta$ -carotene. However, in 2007, San Giovanni *et al.* suggested that consuming 3-6 mg of  $\beta$ -carotene daily will maintain plasma  $\beta$ -carotene levels in the range associated with a lower risk of chronic diseases. Thus, a diet that contains 5mg of *Solanum macrocarpon* and/or *Corchorus olitorius* per day could provide recommended amounts of  $\beta$ -carotene. As provitamin A,  $\beta$ -carotene is the main dietary source of retinol for humans. It has been recommended that intake of some extra  $\beta$ -carotene may be beneficial than vitamin A supplements since excess amounts of the latter could be harmful whilst the former prevents vitamin A deficiency (Zhong *et al.*, 2012). Thus, instead of consuming vitamin A supplements, it is advisable to consume *Solanum macrocarpon* and/or *Corchorus olitorius* since they are rich in  $\beta$ -carotene.

There is epidemiological evidence of a relationship between low plasma concentration of lutein and zeaxanthin and the risk of developing age-related macular degeneration (AMD) on the other hand (Le and Xiao-Ming, 2010). In 2007, San Giovanni and his colleagues of the National Eye Institute, Maryland, USA reported that lutein and zeaxanthin protect against blindness (AMD)

affecting 1.2 million Americans mostly after 65 years. Lutein and/or zeaxanthin are a natural part of human diet when fruits and vegetables are consumed. For individuals lacking lutein and/or zeaxanthin; lutein and/or zeaxanthin-fortified foods are available, or in the case of elderly people with a poorly absorbing digestive system, a sublingual spray is used. However, no credible evidence exists between intake of supplemental or fortified lutein and/or zeaxanthin foods and the risk of AMD and cataract (Chew *et al.*, 2013; Trumbo and Ellwood, 2006). In effect, no RDA currently exists for lutein and/or zeaxanthin, but positive effects have been seen at dietary intake levels of 6-10 mg/day (San Giovanni *et al.*, 2007). Interestingly, lutein and/or zeaxanthin-fortified foods are expensive and thus obtaining lutein and/or zeaxanthin from their natural sources such as *Solanum macrocarpon* and/or *Corchorus olitorius* at a cheap price is advisable.

#### Conclusion

Both green leafy vegetables are rich sources of carotenoids and the total carotenoid content is higher in fresh *Corchorus olitorius* than fresh *Solanum macrocarpon*. Carotenoids content is highest when vegetable is fresh and cooking time is shortened. Consumption of *Solanum macrocarpon* and *Corchorus olitorius* should be encouraged since they are rich in carotenoids that greatly benefit the eyes. Increase consumption of these vegetables will encourage large scale cultivation, which will in turn create employment and income generating opportunities for families, leading to the overall health and wellbeing. Interestingly,  $\beta$ -carotene, lutein and/or zeaxanthin-fortified foods are expensive and thus obtaining  $\beta$ -carotene, lutein and/or zeaxanthin cheaply from these green leafy vegetables: *Solanum macrocarpon* and/or *Corchorus olitorius* are advocated as they also provide dietary fibre which

has implications for good health.

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