

## **Effects of boiling time on the concentrations of vitamin c and beta-carotene in five selected green vegetables consumed in Ghana**

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### **ABSTRACT**

*This study was carried out to determine the effects of boiling on the nutritional composition of selected green vegetables consumed in Ghana. Five green vegetables Cabbage (*Brassica oleracea*), Ayoyo (*Corchorus olitorius*), Okro (*Abelmoschus esculentus*), Abeduru (*Solanum torvum*) and Nkontomire (*Xanthosoma sagittifolium*) were selected. The samples were taken from farms around University of Cape Coast in the Cape Coast Municipality in the Central Region of Ghana. Equal weights of the vegetables were boiled at different boiling times (5, 10, 15 and 20 minutes). The boiled samples were then analyzed for the effect of boiling time on the vitamin C and beta-carotene concentration. The results of the study revealed that as the boiling time increased, the concentration of beta-carotene and vitamin C in the vegetables decreased. Also the reduction in the vitamin C concentration was found to be greater in the vegetables than the reduction in the beta-carotene concentration. It is therefore suggested that boiling of vegetables should be done within the shortest possible time to retain most of these nutrients.*

**Keywords:** *Brassica oleracea, Corchorus olitorius, Abelmoschus esculentus, Solanum torvum, Xanthosoma sagittifolium.*

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### **INTRODUCTION**

In tropical Africa where the daily diet is dominated by starchy staples, African indigenous vegetables are the cheapest and most readily available sources of important proteins, vitamins, especially the pro vitamin A [1] and essential amino acids. Vegetables are the most widely grown crops in Ghana. They provide vital food security for many subsistence farmers in the country. Vegetables rank higher in production than all other crops. They are known to provide 80% of the vitamin A and C in diets [2].

This indigenous knowledge of the health promoting and protecting attributes of African leafy vegetables is clearly linked to their nutritional and non-nutrient bioactive properties. African leafy vegetables have long been, and continue to be reported to significantly contribute to the dietary, vitamin and mineral intakes of local populations [3, 4]. Amaranthus for example is reported to be grown for its leaves which are rich in  $\beta$ -carotene, calcium, iron and vitamin C [1].

The WHO recommended a minimum daily intake of 400 g of fruits and vegetables [5]. However, it is not clear from the report what proportion of this total daily intake should go to vegetables. Nevertheless, according to the Kobe framework document and an FAO report, the recommended total daily intake is equivalent to five servings of 80 g

each of fruits and vegetables [6, 7]. Green vegetables are also great sources of minerals such as zinc, iron and potassium. In recent studies, it is reported that African green vegetables contain non-nutrient bioactive phytochemicals that have been linked to protection against cardiovascular and other degenerative diseases. Nonetheless, Orech *et al.* observed that some of these phytochemicals found in some African leafy vegetables consumed in Western Kenya may pose toxicity problems when consumed in large quantities or over a long period of time. These naturally occurring compounds have attracted great attention from the scientific community for their antioxidant properties and their implication in a variety of biological mechanisms at the base of degenerative processes. Such compounds are secondary plant metabolites responsible for plant food colour, smell, flavour, and bitterness and consist of a wide variety of different molecules, such as carotenoids, polyphenols, vitamins and glucosinolates [8].

Green leafy vegetables like cabbage, lettuce, dandelion, moringa, etc. may be eaten raw, boiled or dried. Perhaps the most common use in all parts of the world is boiled vegetable leaves. This process eliminates potential pathogens, sometimes poisonous or irritating substances are neutralized and spoilage is brought to a halt [1]. In Ghana, most people consume indigenous green vegetables such as cocoyam leaves “Nkontomire”, “Kwansusuaa” Okro, “Ayoyo” and Cabbage. It is known that cooking induces significant changes in chemical composition, influencing the concentration and bioavailability of bioactive compounds in vegetables [1]. However, both positive and negative effects have been reported depending upon differences in process conditions and morphological and nutritional characteristics of vegetable species. Physical properties of vegetables are also greatly affected by heat treatments. Texture and colour are considered very important parameters in the cooking quality of vegetables, and they may strongly influence consumer acceptance of these food items. Changes in texture are often dramatic because of the membrane disruption and the associated loss of turgor. In addition, cooked vegetables exhibit poor colour quality in comparison with fresh ones. Although consumption of fresh unprocessed plant food is widely advocated, evidence is emerging that *in vivo* bioavailability of many protective compounds is enhanced when vegetables are cooked. As much as there are positive attributes of boiling vegetables before consumption, consumers are deprived of important nutrients as the nutritional properties of the vegetables are greatly affected.

This study aims at determining the concentration of vitamin C and beta-carotene of the selected vegetables at different boiling (cooking) times.

## MATERIALS AND METHODS

### Sample collection

Samples of *Xanthosoma sagittifolium*, *Brassica oleracea*, *Corchorus olitorius* leaves, *Solanum torvum* and *Abelmoschus esculentus* fruits for analysis were collected randomly from farms around University of Cape Coast in the Cape Coast Municipality in the Central Region of Ghana. During sampling, well grown green vegetables with no injuries were picked from the stalk of the plants using a kitchen knife.

### Sample preparation

The leaves and fruits collected from the farming site were washed in a basin of water immediately it arrived from the farm. The washed leaves and fruits were rubbed gently with a clean napkin to remove all soil and dust before chopping on a chopping board with a knife. The chopped leaves and fruits were placed in a clean bowl and thoroughly mixed for homogeneity in sampling before treatment.

### Sample treatment

The samples were boiled using a hot plate. About 200 ml of ordinary water was poured into a boiling container and put on the hot plate. As the water in the container began to boil, the chopped homogeneously mixed sample (40 g) was poured into the boiling water in the container and the lid of the container was replaced. At that instant, the timer of a stop clock was started. After five minutes, the boiling was stopped and the sample was taken and put into a clean dry Petri dish and labelled A. The same was repeated for the times of ten minutes, fifteen minutes and twenty minutes and put into a clean dry Petri dish and labelled B, C and D respectively. This procedure was applied to the five different green vegetables that were analyzed. The boiled samples were then ground using mortar and pestle.

### Pigment Extraction for $\beta$ -Carotene analysis

This was carried out according to the method of Association of Official Analytical Chemists [9]. Into a conical flask containing 25 ml of 96.1% ethanol, 5 g of the ground sample were placed and maintained at a temperature of 70-80

$^{\circ}\text{C}$  in a water bath for 20 minutes with periodic swirling. Afterwards, the supernatant was decanted using a suction pump, allowed to cool and its volume was measured by means of a measuring cylinder and recorded as the initial volume. The ethanol concentration was brought to 86.1% by the addition of 15 ml of distilled water and further cooled in a bowl of ice water for about 5 minutes. The mixture was transferred into a 150 ml separating funnel and 25 ml of Petroleum Ether (pet-ether) was added and the cooled ethanol was poured over it. The funnel was corked and swirled gently to obtain a homogenous mixture and allowed to stand until two separate layers were obtained. The bottom layer was run off into a beaker while the top layer was collected into a 250 ml conical flask. The bottom layer collected was transferred back into the separating funnel and re-extracted with 10 ml of pet-ether for 4-5 times until the extract became fairly yellow. The entire pet-ether collected after the extraction was transferred into the funnel for re-extraction with 50 ml of 80% ethanol. The final extract was kept in a conical flask and sealed with an aluminium foil to prevent evaporation of the pet-ether and later analyzed.

#### Extraction for vitamin C analysis

This was carried out using the Colorimetric Analytical method since it gives an accurate analysis of vitamin C content than the other methods of analysis. Into a beaker containing 50 ml of 4% Oxalic Acid solution, 5 g of the ground sample was placed and swirled. The solution containing the ground sample was filtered using filter paper and a funnel over another beaker in order to collect the liquid. An aliquot (10 ml) of the collected liquid was taken using a 10 ml pipette into 50 ml volumetric flask and Bromine water was added drop wise with constant mixing till the extract turned orange-yellow due to excess bromine water. Bubbles were expelled by blowing air into the flask containing the liquid. The volume was made up to the 50 ml mark on the flask with 4% Oxalic Acid solution. Similarly, 10 ml of the stock vitamin C solution was also brominated to obtain an orange-yellow color. A volume of 1 ml of the liquid in the 50 ml volumetric flask was taken using a 1 ml volume pipette into a clean test tube. The volume of the test tube was made to 4 ml by the addition of 1 ml each of Thiourea solution, 2,4-Dinitrophenyl Hydrazine (DNPH) reagent and distilled water. The content of the test tube was thoroughly mixed and incubated in a water bath at  $37^{\circ}\text{C}$  for 3 hours. The solution in the test tube was allowed to cool and 7 ml of 80% Sulfuric acid was added to make up the volume to 11 ml. This was done for all the samples to be analyzed.

#### Determination of $\beta$ -Carotene

The absorbance of the extract was measured using a spectrophotometer (UV mini 1240) at a wavelength of 436 nm. A cuvette containing petroleum ether was used as a blank to calibrate the spectrophotometer to the zero point. Samples of each extract were placed into the cuvette and readings were taken when the figure in the display window became steady. This was done each time after blanking the spectrophotometer with pet-ether. The operation was repeated three times for each sample and the average readings were recorded.

The concentration of the  $\beta$ -carotene was calculated using Beer-Lamberts Law, which states that the absorbance (A) is proportional to the concentration (C) of the pigment as represented by the equation:  $A \propto CL$

$A = ECL$ ;  $C = A/EL$ , where

C = concentration of carotene

A = absorbance

E = extinction coefficient

L = thickness of cuvettes (path length) = 1 cm

E of  $\beta$ -carotene =  $134000 \text{ l mol}^{-1}\text{cm}^{-1}$  [10]

#### Determination of vitamin C

The absorbance of the extract was measured using a spectrophotometer (UV mini 1240) at a wavelength of 540 nm. A cuvette containing the prepared blank solution was used to calibrate the spectrophotometer to the point zero. Samples of the extract were placed into the cuvette and readings were taken when the figure in the display window became steady. The spectrophotometer was blanked each time with the prepared blank solution before the readings were taken. The operation was repeated three times for each sample and the average readings were recorded. The absorbance obtained was extrapolated on a vitamin C standard curve. Each value of the vitamin C obtained from the standard curve was put into the equation below to determine the final concentration.

$$C = [(A \times 1000) / m(V_1 / V_2) \times 1000] \times 50,$$

where

C= concentration of the vitamin C in the sample.

A=concentrations extrapolated from the standard curve.

m=mass of the sample taken (5 g).

V<sub>2</sub>=volume of the extract taken after the filtration (10 ml).

V<sub>1</sub>=volume of oxalic acid used to extract the vitamin C before the filtration (50 ml).

### Statistical analysis

The data obtained were analyzed using SPSS 16.0 statistical tool. All the analyses reported in this study were carried out in triplicates. In each, the mean value and standard deviation were calculated. Analysis of variance (ANOVA) and correlation analysis at  $p < 0.05$  were also performed.

## RESULTS AND DISCUSSION

The results of measurements of Vitamin C and beta carotene of the samples at various boiling times are presented in Figures 1 and 2 respectively.

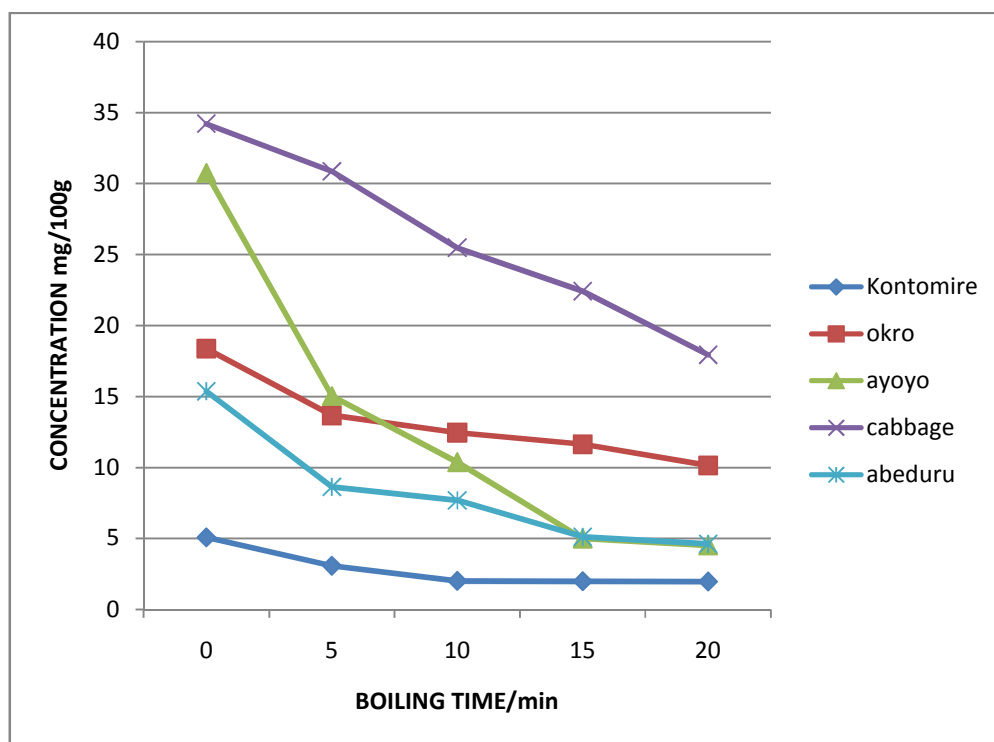


Figure 1: Concentration of vitamin C in the vegetables at the various boiling times.

There is a significant effect ( $p < 0.05$ ) of boiling time on the concentrations of beta carotene and vitamin C in the vegetables, thus increasing boiling time significantly decreases the concentration of beta-carotene and vitamin C. This result corresponds to earlier reports by Oboh and Oboh *et al.* that various conventional food processing methods bring about loss in vitamin C content of leafy vegetables [11, 12].

From the results, similar trend in the effect of the boiling time on the vitamin C and beta-carotene concentration was observed in all the vegetables. From Figures 1 and 2 it was seen that boiling of the vegetables led to the reduction in the beta-carotene and vitamin C concentrations in the various vegetable. There is a significant negative correlation ( $p < 0.05$ ) between the vitamin C concentration and the boiling time of the various vegetables. The reduction in

vitamin C content can be attributed to the fact that it is water soluble and at the same time not heat stable [11]. Also from Figure 1, the highest reduction was observed in the vegetables at the first 5 minutes and 10 minutes and then as the boiling time increased that is, from 15 minutes, there was steady reduction. This can be attributed to the fact that in the initial time of boiling, maximum amount of vitamin C was destroyed or leached into the water hence there was a small amount left in the vegetables during the later boiling times. Also there was the inactivation of enzymes in the initial time of boiling and oxygen was expelled from the water hence deterioration of the nutrients.

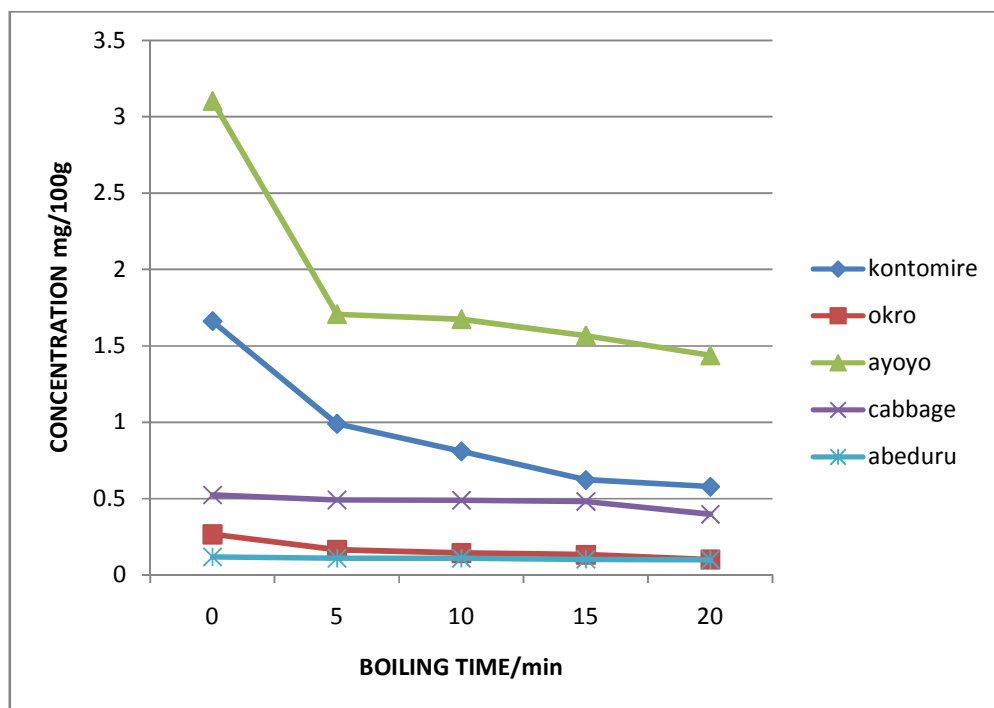


Figure 2: Concentration of beta-carotene in the vegetables at the various boiling times

From the results obtained, the total percentage loss (from 0 to 20 minutes) of vitamin C in the vegetables ranged from 42% to 85%. *Abelmoschus esculentus* was observed to have the least percentage loss of vitamin C content (42.74%) followed by *Brassica oleracea* (47.56%), *Xanthosoma sagittifolium* (52.03%), *Solanum torvum* (69.71%) and *Corchorus olitorius* recording the highest percentage loss (85.33%). This could be attributed to the growth stage of the individual vegetables when the samples were taken.

It can be seen that the concentration of beta-carotene in the vegetables decreased continuously with increase in boiling time (Figure 2). The reduction in beta-carotene content of the vegetables ranged from 16% to 55% after the twenty minutes of boiling. Dietz *et al.* reported that boiling for 30 minutes led to 53% and 40% reduction of carotene in lettuce and carrots, respectively [13]. Anjum *et al.* reported a pronounced reduction in beta-carotene content of selected Indian vegetables [14]. This, however, contradicts the report of Granado *et al.*, that higher levels of carotene in cooked vegetables than in the uncooked vegetables after boiling 18 Spanish vegetables for 10 to 38 minutes [15]. As was observed in the vitamin C, there was a significant negative correlation ( $p < 0.05$ ) between the beta-carotene concentration and the boiling time of the various vegetables indicating that an increase in the boiling time leads to a decrease in the concentration of the beta-carotene concentration in the vegetables. The loss in beta-carotene during boiling was because it is heat-sensitive and also the trans isomer changed to cis isomer as a result of the heat that are biologically inactive [16].

From Figures 1 and 2, it was also observed that the reduction in the vitamin C from one boiling time to the next was found to be higher as compared to the loss in the beta-carotene concentration in the vegetables. This difference can be attributed to the fact that aside vitamin C being heat labile, is also very soluble in water, so some also leached into the water that was used for the boiling unlike the beta-carotene which is not water soluble.

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**CONCLUSION**

The results obtained from the study show clearly that the concentration of both nutrients thus beta-carotene and vitamin C was significantly lowered by boiling time. Increasing or prolonging boiling time caused a continuous decrease in the concentrations of the nutrients with vitamin C experiencing the higher loss. From the results, *Corchorus olitorius* had the highest reduction of 85% in vitamin C, with *Abelmoschus esculentus* also recording the least (42%) reduction in vitamin C among the vegetables. Also, *Abelmoschus esculentus* recorded the highest percentage loss (55.4%) in beta-carotene with *Solanum torvum* recording the least percentage loss (16.5%).

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