Effect of Cooking Time on the Ascorbic Acid Content of Some Selected Green Leafy Vegetables

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ABSTRACT
Cooking is one factor which mainly affects the amount of ascorbic acid in vegetables. The study was aimed at determining the effect of cooking time on the concentration of ascorbic acid in five commonly consumed green leafy vegetables of Shillong city. The concentration of ascorbic acid in the samples was determined by titrimetric method using 2, 6 dichlorophenol indophenol solution. Cabbage contained the highest concentration of ascorbic acid at 119.02mg/100g while the lowest concentration was observed in Spinach at 20.26mg/100g. The effect of cooking on percentage reduction of ascorbic acid was also investigated. A great percentage of it is lost during boiling of vegetables because it is a water-soluble vitamin. The results also showed that there is a rapid degradation of ascorbic acid on boiling from 5 mins to 20 mins. Preventive measures should be taken to avoid the loss of this essential vitamin like boiling only for a very short time.

Introduction
Vitamins are complex biochemicals essential for normal functioning of the human body. One of the essential vitamins that is well known is Vitamin C or L-ascorbic acid. It is a 6-carbon lactone and is characterized by the ene-diol group, which makes it a strongly reducing compound. The D form has no biological activity. Most plants and animals have the ability to synthesize their own Vitamin C from glucose and galactose. But species such as Guinea pigs, apes and humans lack the enzyme L-gulonolactone oxidase, which is necessary for the conversion. Therefore such species must obtain vitamin C in their diet.

There are numerous important roles played by vitamin C in the human body. It is important for the healing of wounds, burns and broken bones as it is required for the synthesis of all connective tissues (Heimann, 1980). It is used in the synthesis of collagen, the protein used to repair damaged tissue. As an antioxidant, the primary role of Vitamin C is to neutralize free radicals. Since ascorbic acid is water soluble, it can work both inside and outside the cells to control free radical damages. Vitamin C is the least stable of all vitamins and is easily destroyed during processing and storage. Exposure to oxygen, prolonged heating in the presence of oxygen and exposure to light are all harmful to the vitamin C content of foods. Because of its vulnerability to oxidation, vitamin C can be destroyed in many different ways. It is water soluble and can easily be leached out during washing or cooking. Therefore regular consumption of fresh fruits and vegetables is the best way to obtain a good source of vitamin C. There has been a vigorous debate on the optimum daily intake of vitamin C. Some have argued that 200mg/day is an optimal daily intake for adult humans. Others have suggested 1-2 g/day is best despite studies that show that the blood is saturated with vitamin C at 100mg/day, and any excess is excreted in the urine. In an attempt to balance the competing claims and ensure the general population’s good health, the Federal Food and Drug Administration has adopted a recommended dietary allowance (RDA) of 60mg/day for adults (aged 15 or older), less for children, and more for pregnant and lactating women. Fruits, vegetables, and organ meats (e.g., liver and kidney) are generally the best sources of ascorbic acid; muscle meats and most seeds do not contain significant amounts of ascorbic acid. The amount of ascorbic acid in plants varies greatly, depending on such factors as the variety, weather, and maturity. But the most significant determinant of vitamin C content in foods is how the food is stored and prepared. Since vitamin C is easily oxidized, storage and the cooking in air leads to the eventual oxidation of vitamin C by oxygen in the atmosphere. In addition, its water-solubility means that a significant amount of vitamin C present in a food can be lost by boiling. The study aims to determine the effect of cooking and the duration of exposure to heat on ascorbic acid content in green leafy vegetables commonly consumed by the people of Shillong city in Meghalaya state, India as these vegetables are often over heated in the process of cooking.

Materials and methods
Sample collection and preparation:
Five commonly consumed fresh green leafy vegetables namely Mustard leaves (Brassica campestris), Cabbage (Brassica oleracea), Raddish leaves (Raphanus sativus), Spinach (Spinacea oleracea), and Beet leaves (Beta vulgaris) were purchased from the local market of Shillong city of Meghalaya. The samples were first thoroughly washed with distilled water to remove all the dirt and dust particles. The chopped vegetables were placed in a clean bowl and thoroughly mixed for homogeneity in sampling before treatment.

Sample treatment:
200ml of ordinary water was poured into a beaker and heated on the hot plate. As the water in the beaker began to boil, 20 grams of the chopped sample was poured into the boiling water in the beaker. Each sample was subjected to boiling for 5 mins, 10 mins, 15 mins and 20 mins. The boiled samples were then ground using mortar and pestle. Vitamin C concentration of the samples was determined immediately to counteract its instability.

Determination of Ascorbic Acid
The method described by Sadasivam and Manickam(1996) was employed. The method devised to determine vitamin C is the redox titration with 2, 6-dichlorophenol indophenol (DCIP). The oxidation of ascorbic acid yields dehydroascorbic acid. This re-dox reaction is a redox reaction in which vitamin C (ascorbic acid) is oxidized to dehydroascorbic acid and DCIP is reduced to the colorless compound DCIPH2. DCIP is blue in neutral solution and pink in acidic solution. The reduced form is colourless, so the endpoint of the titration is the appearance of a faint pink colour. All reagents used in the analysis were of analytical grade and all titrations were done in triplicates.
The vitamin C present in the samples was extracted with 10% oxalic acid and thereafter determined by titrimetric method using 2, 6- dichlorophenol indophenol dye. The method involved preparing a stock standard solution and a working standard solution. The stock standard solution was prepared by dissolving 100mg ascorbic acid in 100ml of 4% oxalic acid solution. The working standard solution was prepared by diluting 10ml of the stock solution to 100ml with 4% oxalic acid (100μg/ml). From this solution 5ml was pipette into a 100ml conical flask and 10ml of 4% oxalic acid was added. This was titrated against the dye and the end point was indicated by the appearance of a pink coloration that persisted for a few minutes. The amount of dye consumed is equivalent to the amount of ascorbic acid. Then 5g of the sample was extracted and made up to 100ml volume using 4% oxalic acid and centrifuged. 5ml of the supernatant was pipette and 10ml of 4% oxalic acid was added and titrated against the dye and the volume was recorded. Ascorbic acid content was expressed in mg/100g of the sample.

DISCUSSION

Vitamin C is highly sensitive to oxidation and is easily destroyed on heating and storage at higher temperatures. The chemical instability of vitamin C acid is due to the fact that it is a strong reducing agent and can be deactivated by a wide range of oxidizing agents\(^7\). The effect of boiling on the vitamin C contents of five green leafy vegetables is shown in Figure 1. The highest concentration of vitamin C was found in cabbage at 119.02mg/100g and the lowest concentration was determined in spinach at 20.26mg/100g. The effect of boiling on the percentage reduction of vitamin C at different temperature is shown in Figure 2. Cooking time really affected the percentage of vitamin C significantly. The results showed that there is a rapid degradation of vitamin C concentration on heating from 5mins to 20mins. It was observed that the highest loss of ascorbic acid was seen in the case of mustard leaves with a reduction of 67.86% on boiling at 5mins and also to the head of the department of chemistry for allowing the Principal of Lady Keane College for carrying out the project. The authors gratefully acknowledged the financial support from the Principal of Lady Keane College for carrying out the project.

The result showed that the longer the boiling time and the longer the duration of exposure to heat the more the loss of ascorbic acid. The reduction in vitamin C content of cooked vegetable is in agreement with the study conducted by Oboh (2005) on the effect of blanching on the vitamin C content of some green leafy vegetables\(^6\). Adefega and Oboh, 2011 also attributed that the loss of vitamin C content during the different cooking methods could be due to the fact that vitamin C is highly soluble in water and is very unstable at high temperatures\(^8\). Cooking temperature is one such factor which dramatically affects the nutrient retention. Oke\(^11\) reported the changes in vitamin C content during various stages of growth of some Nigerian leafy vegetables. Krehl et al\(^12\) and Fafunso et al\(^13\) reported that vitamin C is also reduced by cooking. The loss of vitamin C observed when the vegetables were subjected to heat are in agreement with the work of Babalola et al (2010) who confirmed that ascorbic acid levels in green leafy vegetables are temperature dependent\(^14\). Losses of antioxidant components from vegetables during cooking have also been reported\(^15, 16,\) and 17\). The main finding of this study that Vitamin C is unstable at high temperatures is also in agreement with studies by Nagy et al\(^18\), Lyimo et al\(^19\) and Lee et al\(^20\). The losses observed in this study are very high especially when the vegetables were subjected to boiling for a longer duration. Egerg et al\(^21\) reported that the loss as a result of boiling is justified since vitamin C is water-soluble and heat labile.
REFERENCE