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# Analysis of Vitamin C (ascorbic acid) Contents in Various Fruits and Vegetables by UV-spectrophotometry

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## Abstract

Total vitamin C (ascorbic acid + dehydroascorbic acid) has been determined by UV-spectrophotometric method in various fruits and vegetables. In this method bromine water oxidizes ascorbic acid to dehydroascorbic acid in presence of acetic acid. After coupling with 2,4-dinitrophenyl hydrazine at $37^{O}$C temperature for three hours, the solution is treated with 85% $H_2SO_4$ to produce a red color complex and the absorbance was spectrophotometrically measured at 521 nm. The content of vitamin C were 10 mg/100g to 80 mg/100g in fruits and 16 mg/100g to 42 mg/100g in vegetables. A loss of 64% at $-10^{O}$C and 76% at $5^{O}$C was observed after two months storage period of one leafy vegetable "*Enhydra fluctuans*" (Helencha shak).

**Key words:** Vitamin C determination, Vegetables, Fruits, UV-spectrophotometer.

## Introduction

Vitamin C is one of the most important vitamin for human nutrition that is supplied by fruits and vegetables. L-Ascorbic acid (AA) is the main biologically active form of vitamin C. Ascorbic acid is reversibly oxidized to form L-dehydroascorbic acid (DHA), which also exhibits biological activity. Since DHA can be easily converted into AA in the human body it is important to measure both A and DHA in fruits and vegetables to know vitamin C activity (Lee and Kader, 2000). Among the vitamins, vitamin C (ascorbic acid) (ascorbic acid) is an essential micronutrient required for normal metabolic function of the body ( Jaffe, 1984). Vitamin C is easily oxidized, and the majority of its functions in vivo rely on this property. The human body cannot produce ascorbic acid, and so it must be obtained entirely through one's diet. A vitamin C deficiency in humans results in the disease called scurvey, whose symptoms include hemorrhaging, joint pain and exhaustion (Brody, 1994 and Pauling, 1976). A very small daily intake of vitamin C (10-

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15 mg/day for an adult) is required to avoid deficiency and stave off scurvey (Kallner, 1986).

Vitamin C is the major water-soluble antioxidant within the body (Sies, *et al.,* 1995; Levine, 1986; Levine, 1995). It lowers blood pressure and cholesterol levels ( Rath, 1993). Numerious analysis have shown that an adequate intake of vitamin C is effective in lowering the risk of developing cancers of the breast, cervix, colon, rectum, lung, mouth, prostate and stomach (Levine, 1996; Block, 1992; Block, 1991). Vitamin C is generally non-toxic. For maintaining a good and sound health and for prevention from common cold, human body should be kept saturated with vitamin C. Keeping in view its importance, the estimation of Vitamin C containing this vitamin assumes significance.

An accurate and specific determination of the nutrients content of fruits is extremely important to understand the relationship of dietary intake and human health. A wide variety of food exists that contains vitamin C. Fruits, vegetables, and organ meats are generally the best sources of ascorbic acid; muscle, meats and most seeds do not contain significant amounts of ascorbic acid (Combs, 1992). For better utilization of fruits and vegetables as a human food, clear understanding of their nutrition value as well as the content of vitamin C estimation is essential. A variety of citrus fruits are available in tropical Bangladesh. The amount of ascorbic acid in plants varies greatly, depending on such factors as the variety, weather and maturity (Chaney *et al*., 1979). Vitamin C content of some fruits and vegetables available in Bangladesh, has been reported by Biswas *et al.* (Biswas and Mannan 1996). There are many citrus fruits and vegetables such as Helencha, Pudina leaf, Punonnoma etc available in different region of Bangladesh and vitamin C content of these fruits and vegetables are not known as per our knowledge. Recently we have reported the estimation of vitamin C content of locally available some citrous fruits by 2,4-DNPH method (Rahman *et al.* 2005; Khan *et al.* 2006). As a part of our ongoing study on vitamin C estimation we have collected more fruits and vegetables from different district of Banglsdesh and estimate their vitamin C content. In this paper we would like to describe the results of these samples.

**Materials and Methods**

Several analytical methods have been reported for the determination of vitamin C such as titrimetry (Kabasakalis, 2000), biological, electrochemical, and chromatographic method (Arya, *et al.*,1998; East *et al.*, 2002; Geigertj *et al*., 1981; Veasey *et al.*, 1980). All the method has great limitation in use for different purpose. It is very difficult to choose a unique method for determining the content of total vitamin C in food products, biological samples and pharmaceuticals. Because

each sample has its own specific characteristics and properties in terms of extraction, purification, interference of other compounds (such as color, presence of oxidizing and reducing components etc).

Although some methods are available for determination of ascorbic acid but very few methods are employed for the determination of both forms (ascorbic acid and dehydroascorbic acid) of ascorbic acid. This is because two forms of the vitamin C, ascorbic acid and its oxidized form dehydroascorbic acid possess the different chemical, optical and electrochemical properties. For example, the AOAC's official method (Kabasakalis, 2000) based on the titration of AA with 2,6-dichloroindophenol in acidic solution is not applicable in all the matrices. Substances naturally present in fruits such as tannins, betannins, Cu(II), Fe(II) and Co(II) are oxidized by dye. Moreover, the method is applicable only when the concentration of DHA is low.

To determine the content of total vitamin C in food samples, here a well-established chemical method was used (Riemschneider *et al*., 1976) as a simplified method for the simultaneous determination of the total vitamin C.

**Instrument**

A Shimadzu spectrophotometer (model UV-1601) with a pair of 1 cm quartz cells was used.

**Chemical and Reagent required**

**5% Metaphosphoric acid-10% acetic acid**

15g of solid metaphosphoric acid (E. Merck) were dissolved in a mixture of 40 ml of glacial acetic acid (BDH) and 450 ml of distilled water in a 500 ml volumetric flask. The solution was filtered and collected.

**10% Thiourea solution, 2,4-Dinitrophenylhydrazine solution, 85% Sulphuric acid**

**Standard vitamin C ( ascorbic acid) solution**

Stock standard solution containing 0.5 mg/ml of ascorbic was prepared in water by dissolving 0.05 g of AA in 100 ml of water and stored in a glass stoppered bottle. Solutions of variable concentrations were prepared by diluting the stock solution in water.

**Sample preparation**

10 g blended sample was homogenized with about 50 ml of 5% metaphosphoric acid-10% acetic acid solution. Then it was quantitatively transferred into a 100 ml volumetric flask and was shaken gently until a homogeneous dispersion was obtained. Then it was diluted up to the mark by the 5% metaphosphoric aceid-10% acetic acid solution. Then the solution was filtered and the clear filtrate was collected for the determination of vitamin C in that sample.

**Estimation of vitamin C**

**Procedure**

Bromine water were added to the filtered sample solution to oxidize the ascorbic acid to dehydroascorbic acid. Then a few drops of thiourea was added to it to remove the excess bromine and thus the clear solution was obtained.

Standard solutions of ascorbic acid (5 ppm, 10 ppm, 15 ppm, 20 ppm and 25 ppm) were prepared from 500-ppm stock solution of ascorbic acid by proper dilution. Then 1 ml of 2,4- DNPH solution was added thoroughly with all standards and also with the oxidized ascorbic acid. For the completion of the reaction, all the standards, samples and blank solution were kept at $37^{O}$C temperature for 3 hours in a water bath (thermostatic). After this incubation all of those were cooled in an ice bath and treated with 5 ml of 85% $H_2SO_4$ with constant stirring. As a result, a colored solution was obtained whose absorbance was taken at 521 nm.

**Reactions:** a. Ascorbic acid is oxidized to dehydroascorbic acid by the action of bromine solution.

b. L-dehydroascorbic acid reacts with 2,4-dinitrophenylhydrazine and produces an osazone which on treatment with 85% $H_2SO_4$ forms red colored solution.

**Reproducibility**

The reproducibility of this method was checked by determining the % recovery of known amount of vitamin C from a sample. This can be done by addition of different known amount of vitamin C in this sample. For instance, the concentration of the sample is X ppm and then 5 ppm standard is added and the observed concentration of the mixture is X' then the % recovery is given by

$$\% \text{ recovery} = (\text{observed conc.} / \text{calculated conc.}) \times 100\%$$
$$= X' / (X+5) \times 100\%$$

## Results and Discussion

**Calibration curve**

After determination of the $\lambda_{max}$ of the colored complex (521 nm) using a Shimadzu UV- spectrophotometer the absorbance of the all standards (converted to coloured complex) were taken to construct a calibration curve. The calibration curve was constructed by plotting the concentration versus the corresponding absorbance. Molar absorptivity was found 0.0328 L $mol^{-1}$ $cm^{-1}$ using Beer - Lambert plots (Fig. 1).

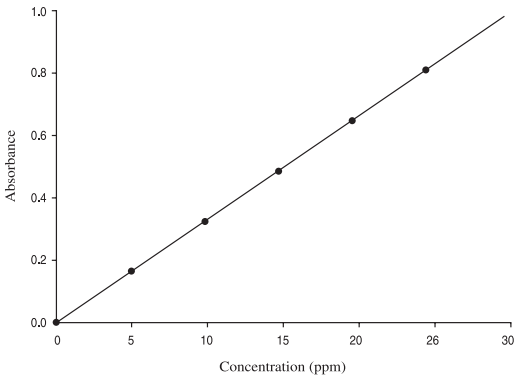


**Fig. 1. Calibration curve of standard vitamin C at 521 nm**

**Determination of vitamin C in samples**

Generally all the parts of a fruit and all fruits have not equal amounts of edible part. In the comparative study of the vitamin C content in various fruits and vegetables, the percent of edible parts of those must also be considered. Fruits such as orange, lemon etc contain high amount of vitamin C and vegetables such as Cabbage, Green pepper, Red pepper also have relatively high amount (7 mg/100g to163 mg/100g) vitamin C.23 In mg/100gm and Sofeda contain 21.72 mg/100gm of vitamin C. By the similar methods vitamin C content of the fruits Shaluk, Chinichampa and vegetables such as Punonnoma shak, Helencha shak, sweet potato leaf, Pudina leaf etc has been determined. Results are summarized in Table I.

The ascorbic acid concentration vary with conditions such as temperature and the storage period on peservation. Therefore, changes of ascorbic acid content at one selected vegetables such as Helencha shak during frozen storage at different conditions (two months storage at different temperature) were also studied. It is observed that vitamin C content slowly decreases with temperature and storage period of vegeta-

**Table I. Total vitamin C content in fruits and vegetables**

| Serial No. | Samples | Botanical name | % of edible part | Condition | Temperature ( $^{O}$C) | Total vitamin-C (mg/100g) | Percent of standard deviation (%S) |
|---|---|---|---|---|---|---|---|
| 1 | Punonnoma shak (Hogweed) | *Boerhavia diffusa* | 80 | Fresh | Room temp. | 16.512±0.434 | 2.629 |
| 2 | Shapla(Water Lilly) | *Nymphaea noucifera* | 85 | Fresh | r.t | 97.477±0.759 | 0.779 |
| 3 | Sofeda | *Manilkara zapota* | 80 | Fresh | r.t | 21.719±0.063 | 0.291 |
| 4 | Chinichampa (Banana) | *Musa spp* | 90 | Fresh | r.t. | 69.567±0.936 | 1.346 |
| 5 | Jackfruit Muchi | *Artocarpus heterophyllus* | 98 | Fresh | r.t. | 80.067±0.251 | 0.313 |
| 6 | Talakuchi | - | 95 | Fresh | r.t. | 29.615±0.071 | 0.238 |
| 7 | Sweet potato leaf | *Ipomoea batatus* | 95 | Fresh | r.t. | 42.671±0.117 | 0.274 |
| 8 | Shaluk | *Nymphaeanouchali* | 95 | Fresh | r.t. | 10.189±0.09 | 0.885 |
| 9 | Laboi | - | - | Fresh | r.t. | 31.274±0.165 | 0.530 |
| 10 | Palong shak (Spinach) | *Spinacea oleracea* | 95 | Fresh | r.t. | 21.439±0.153 | 0.716 |
| 11 | Leaf of Pudina(Mint) | *Mentha longifolia* | 95 | Fresh | r.t. | 14.524±0.424 | 2.921 |

bles ( Table II). The initial concentration of ascorbic acid was 31mg/100g and decreased by 76% at 5$^{O}$C and 64% at -10$^{O}$C during two months storage time. Boil at higher temperature and frying drastically reduce the ascorbic acid. The results are shown in Table II.

The reliability of this method is justified by the calculation of the % of standard deviations and it was found to be varied within the range from 0.08 % to 3.24 % (Table I). The reliability of this method is also confirmed from the consideration of the following expected interferences.

**i) Interferences due to diketogulonic acid**

Due to the destructive oxidation hydrolysis at higher pH results the opening of the

**Tabel II. Effect of preservation and cooking of Helencha shak (*Enhydra fluctuans*)**

| Sample | Botanical name | Edible part (%) | Condition | Temperature ( $^{O}$C) | Days | Total vitamin- C (mg/100g) | Percent of standard deviation (%S) |
|---|---|---|---|---|---|---|---|
| Helencha shak | *Enhydra fluctuans* | 95 | Fresh | Room temp. | | 34.554±0.107 | 0.310 |
| | | | Preservation | 5$^{O}$C | 7 | 30.067±0.09 | 0.300 |
| | | | | | 15 | 25.914±0.048 | 0.188 |
| | | | | | 21 | 22.335±0.141 | 0.633 |
| | | | | | 30 | 18.146±0.059 | 0.329 |
| | | | | | 37 | 14.518±0.019 | 0.134 |
| | | | | | 45 | 11.365±0.031 | 0.278 |
| | | | | | 52 | 8.426±0.039 | 0.463 |
| | | | | | 60 | 4.835±0.039 | 0.807 |
| | | | | -10$^{O}$C | 7 | 32.658±0.080 | 0.246 |
| | | | | | 15 | 30.347±0.026 | 0.088 |
| | | | | | 21 | 27.237±0.058 | 0.214 |
| | | | | | 30 | 24.829±0.026 | 0.108 |
| | | | | | 37 | 22.567±0.053 | 0.237 |
| | | | | | 45 | 19.615±0.026 | 0.136 |
| | | | | | 52 | 17.945±0.051 | 0.285 |
| | | | | | 60 | 15.847±0.041 | 0.261 |
| | | | Boil (in 80 ml water for 20 min) | 85$^{O}$C | | 6.890±0.073 | 1.061 |
| | | | Fry | 10 min | | 23.231±0.048 | 0.209 |
| | | | | 20 min | | 13.487±0.051 | 0.379 |
| | | | | 30 min | | 11.347±0.068 | 0.601 |

lactone ring of the ascorbic acid and loose the vitamin activity. These processes are naturally occurred in fruits and some amounts of diketogulonic acid is present in the fruits. (Kabasakalis *et al.*, 2000). As the diketogulonic acid has keto group, it should give the osazone with DNPH as that of ascorbic acid and should give the colored complex on treatment with 85% $H_2SO_4$. Thus there is chance of error in this method. But actually this cannot interfere with the ascorbic acid.

Here diketogulonic acid was prepared by the acid hydrolysis (dilute $HNO_3$) of ascorbic acid. The spectrum shows that there is no considerable absorption peak near the 521 nm (the absorption maxima of DNPH complex of ascorbic acid).

**ii) Interference due to extracted glucose:** As ascorbic acid is largely similar to the glucose by structure, some of glucose may be extracted in the meta-phosphoric acid during the extraction of ascorbic acid from sample. Because of their structural similarity, glucose may also form the colored complex with DNPH as ascorbic acid. But actually no such interference is occurred which is evident from the following spectrum are given in fig-2.

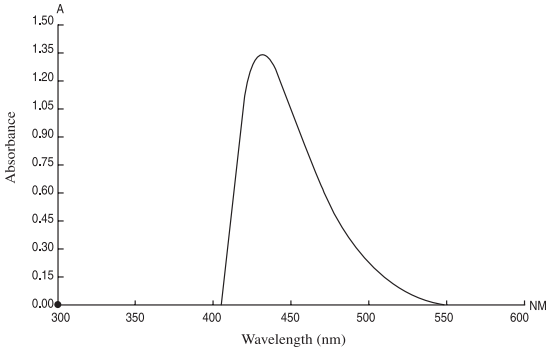


**Fig. 2. Spectrum of DNPH complex of glucose**

From the spectrum it is evident that there is no absorption peak around the interested peak at 521 nm.

## Conclusion

The locally available some fruits and vegetables are a valuable and natural sources of vitamin C. The method is simple and offers an excellent method for the determination of total vitamin C in fruits and vegetables.

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