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ANALYSIS OF TOTAL VITAMIN C CONTENTS IN VARIOUS FRUITS AND VEGETABLES BY UV-SPECTROPHOTOMETRY

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Abstract

In the present study, a simple UV- spectrophotometric method for the determination of the total vitamin C (ascorbic acid + dehydroascorbic acid) in various fruits and vegetables is described. In this method bromine water is added which oxidizes the ascorbic acid into dehydroascorbic acid. After coupling with 2,4 -dinitrophenyl hydrazine at 37°C temperature for about three hours, the solution is treated with 85% H_2SO_4 to produce a red color complex. Then, the absorbance was spectrophotometrically measured at 521 nm. The content of vitamin C was 1.868 to 51.74 mg/10g in fruits and 0.841 to 17.416 mg/10g in vegetables. The standard deviation and the possible interfering factors are also discussed.

Keywords: *Ascorbic acid, UV spectrophotometer; total vitamin C, 2,4-dinitrophenylhydrazine, spectrophotometric method, fruit and vegetables.*

Introduction

Human health is very important to our survival. Vitamins help the human to maintain a healthy diet. They serve as essential components of the specific coenzymes participating in metabolism and other specialized activities. Vitamin C is one of the most important vita-min for human nutrition that is supplied by fruits and vegetables. L-Ascorbic acid (AA) is the main biologically active form of vita-min 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 [1](Lee and Kader, 2000). Among the vitamins, vitamin C (ascorbic acid) is an essential micronutrient required for normal metabolic function of the body[2](Jaffe, 1984). Human and other primates have lost the ability to synthesize vitamin C as a result of a mutation in the gene coding for L-gulonolactone oxidase, an enzyme required for the biosynthesis of vitamin C via the glucuronic acid pathway[3] (Woodall & Ames, 1997). 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[4,5] (Brody, 1994 and Pauling, 1976).

A very small daily intake of vitamin C (10-15 mg/day for an adult) is required to avoid deficiency and stave off scurvy[6] (Kallner, 1986).Our bodies need vitamin C to make a substance collagen which is important for the health and repair of our skin, bones, teeth and cartilage. Vitamin C is the major water-soluble antioxidant within the body[7,8,9] (Sies, *et al.*, 1995; Levine, 1986; Levine, 1995). It lowers blood pressure and cholesterol levels[10](Rath, 1993). Numerous analysis has 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[11,12,13] (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 contain-ing this vitamin assumes significance.

Vitamin C, in collaboration with vitamin E, plays an important role in the protection of cell from oxidants attack, where ascorbic acid reversibly oxidized to dehydroascorbic acid, scavenges many types of free radicals and regenerates the reduced form of α-tocopherol [14,15,16].

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. The common sources of vitamin C are citrus fruits and some other foods like tomatoes, broccoli, cauliflower, spinach, ladyfinger etc. The development of rapid, simple, and inexpensive analytical methods is one of areas of growing interest, especially since quick decisions are needed in environmental, medical, and industrial fields.

Direct spectrophotometry has not found widespread routine application in the determination of ascorbic acid in foods, owing to the rigorous sample preparation that would be required to obtain a sufficiently pure solution for assay [17]. In addition, both the titrimetric (DCPIP) and spectrophotometric (DNP) methods are time consuming. The DNP method often produces errors, partly because only 85% of dehydroascorbic acid reacts with DNP at 37ºC over a 3-hours period, and slight fluctuations of incubation temperature and time affect the data [18]. However, the assay has the advantage of being simple, making the technique widely accessible and reproducible, in comparison with the titrimetric method. One of the major concerns in using the spectrophotometric procedures for estimation of vitamin C in foods is the requirement of several chemicals, some of them are harmful in handling and exert toxic properties, such as the use of bromine, cyanogen bromide and other bromine derivatives, being very poisonous and must be handled with caution. Moreover, the method is of limited use for the highly colored solutions, unless proper measures are taken [19].

The spectrophotometric procedure presented herein for the estimation of total ascorbic acid was originally developed by Roe and Kuether[20] for the estimation of the vitamin C content in biological fluids such as blood and urine. The procedure was widely used for that purpose because of its simplicity, reproducibility, precision and of less use of hazardous chemicals. The assay has found limited application in the determination of vitamin C in food products, mainly because of the rigorous sample preparation that would be required to obtain a pure solution for assay [21]. Therefore, modifying the method of sample preparation of food materials will make this DNP procedure as one of the most simple, accurate and applicable methods for the assay of total ascorbic acid in fresh foods such as fruit and vegetables. Although the method is less sensitive than HPLC procedure, it is however easy to use in conventional research laboratories.

Description of the 2,4-dinitrophenylhydrazineSpectrophotometric Method

The visual titration method [22] to determine vitamin content of fresh produce is based on the reduction of the dye 2,6-dichlorophenolindophenol by the acid solution of ascorbic acid. The method is applicable to the determination of reduced form of ascorbic acid (L-ascorbic acid) only, and not for the total vitamin C (ascorbic acid plus dehydroascorbic acid). It is not applicable for samples containing ferrous, stannous, cuprous, sulfate, thiosulfate ions or SO_2. The end point is difficult to detect with colored samples, and thus producing inaccurate results [23]. However, sample preparation of fruit and vegetables does not require the complicated procedure needed for other methods.

The 2,4-dinitrophenylhydrazine (DNP) method [21] used in the present study is widely applied for the determination of total ascorbic acid in biological fluids such as blood and urine. The blood samples are simply left to clot and centrifuged to get the serum for analysis. Alternatively, the blood samples are collected in heparinized tubes and centrifuged to obtain plasma samples that are ready for the estimation of total ascorbic acid content. Ascorbic acid is oxidized by Cu(II) to form dehydroascorbic acid and then to 2,3-diketo-gulonic acid, which reacts with 2,4-dinitrophenylhydrazine to form a red bis-hydrazone, which is measured at 520 nm wavelength.

In order to apply the same DNP method for the estimation of total vitamin C in fruit and vegetables, preparation of the sample for assay is the most detrimental step in the whole procedure. In the titration method, the determination of vitamin C in fruit and vegetable samples requires only preparation and homogenization with metaphosphoric acid, centrifugation and normal filtration to obtain the proper solution ready for titration. Such solution will not be applicable for the colorimetric procedure, where the result is often fluctuating and non-reproducible, and therefore requires further important measure. In order to avoid such obstacle and to get reproducible results, we have found that using vacuum micro-filtration with cellulose nitrate membrane Whatman filter paper 0.45 µm or 0.20 µm is an essential last step for getting a clear stable ready-to-use supernatant solution. The final solution obtained is then used for spectrophotometric estimation of vitamin C content of the food material.

Materials and Extraction Procedure

A detailed description of the reagents and generalized experimental procedure has been presented by McCormick and Greene [21]. The procedure is an adaptation of the original method described by Roe and Kuether[20], based on the oxidation of ascorbic acid into dehydroascorbic acid and diketogulonic acid, followed by coupling with 2,4-dinitrophenylhydrazine under carefully controlled conditions to give red-colored osazones. A comparison of color produced in samples and ascorbic acid standard solutions is used as means of determining ascorbic acid content [5].

In brief, the suggested DNP method involves the following steps:

(1) Extract food samples and homogenize with freshly prepared6% (6.0 g/dL) metaphosphoric acid, centrifuge at 6,000 x g for 10 minutes at 4°C and filter using an ordinary Whatman No. 1 filter paper followed by further filtration using 0.45 µm or 0.2 µm

filter paper for the final working solution. Both filtrations were carried out under vacuum conditions.

(2) Using a volumetric flask, prepare 25 mL of working calibrators of standard ascorbic acid solutions of each of the concentrations: 0.10, 0.40, 0.80, 1.20, 2.0, 3.0 and 4.0 mg/dL using 6% metaphosphoric acid. [For conversion of conventional units (mg/dL) to SI units (mmol/L) multiply by 56.78] [24].

(3) Pipette triplicate samples of 1.2 mL of both the clear supernatant extract and the working calibrators into 13 x 100 mm Teflon-lined screw–cap test tubes. Place 1.2 mL of the 6% metaphosphoric acid into two separate tubes for use as blanks.

(4) Add 0.4 mL of dinitrophenylhydrazine-thiourea-copper sulfate (DTCS) reagent to all tubes. Cap, mix the contents, and incubate tubes in a water bath at 37ºC for 3 hours.

(5) Remove the tubes from the water bath and chill for 10 minutes in an ice bath. While mixing slowly, add to all tubes 2.0 mL of cold sulfuric acid (12 mol/L), cap and mix in a vortex mixer (the temperature of the mixture must not exceed room temperature).

Adjust the spectrophotometer with the blank to read zero at 520 nm and read the calibrators andunknowns. Plot the concentration of each working calibrator versus absorbance values. The calibration curve obeys Beer's law up to an ascorbic acid concentration of 2.0 mg/dL.

Measurement of Vitamin C Content in Fresh Fruit

Materials and methods: To verify the application of the 2,4-dinitrophenylhydrazine spectrophotometric method for fresh fruit and vegetables, experiments were carried out to determine the vitamin C contents of selected types of fruit. For comparison, the vitamin C contents of sub-samples were also determined by the traditional titration method [22]. Samples of Star gooseberries, mulberry, karanda, black current, grapes, mango, guava, wax apple, muskmelon, watermelon, pomegranate, lemon (sweet lemon) were analyzed. Samples were purchased fromlocal market.

Result and Discussion

Calibration curve and vitamin C content of fruit: Graphical plots of concentration of each working calibrator versus absorbance values obtained showed that the calibration curve followed Beer's law. A typical calibration curve obtained in the present study is shown in Fig. 1. It was found that for fresh produce juice, these relationships were particularly valid up to ascorbic acid concentration of 2.0 mg/dL, which is typical of fruit samples.

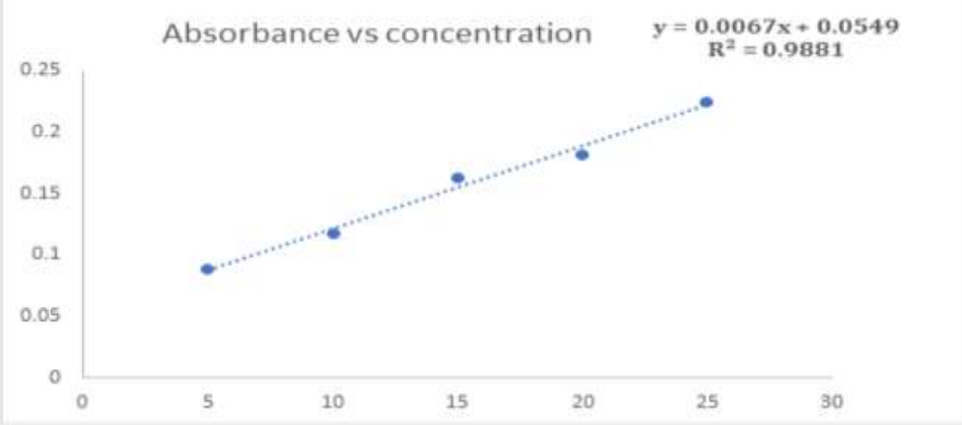

Figure 1: Calibration curve of ascorbic acid.

Determination of vitamin C using UV-Spectrophotometer Inthisworkfordetermination of vitamin C in fruits, fruits were fresh and collected from local market. Black current shows maximum amount of vitamin C and grapes shows minimum amount of vitamin C (Table 1, Figure 2).

Table: 1

S. No.	Sample	Biological Name	Amount of Vitamin C (mg/100gm)
1.	Mulberry	*Morus Nigra*	41.8
2.	Pomegranate	*Punica granatum*	8.22
3.	Watermelon	*Citrullus lanatas*	9.79
4.	Karanda	*Carissa carandas*	44.02
5.	Mango	*Magnifera indica*	36.41
6.	Black Current	*Ribes nigrum*	505.60
7.	Mango	*Magnifera indica*	35.67
8.	Lemon	*Citrus limetta*	72.62
9.	Muskmelon	*Cucumis melo*	44.73
10.	Wax apple	*Syzygiumsamarangense*	16.28
11.	Guava	*Psidium gujava*	202.76

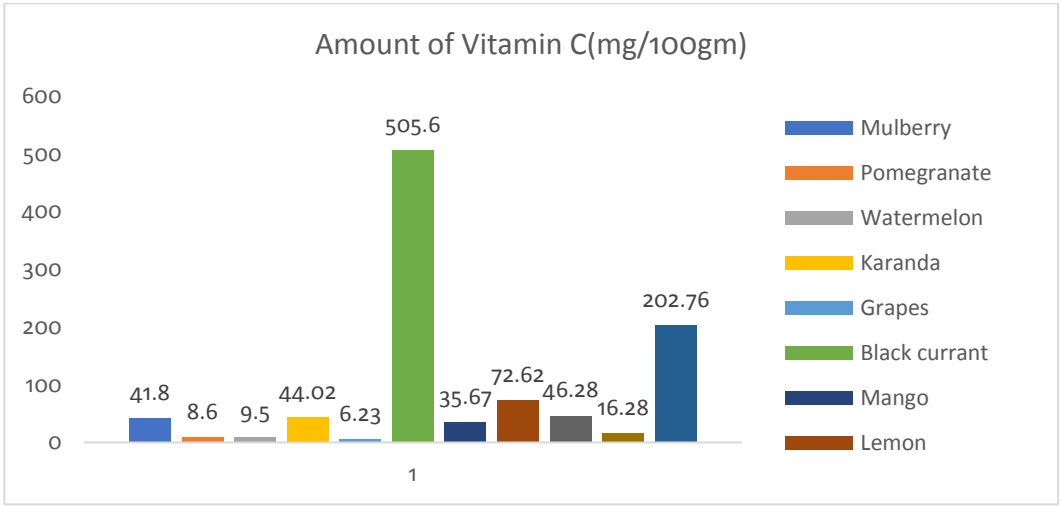

Figure 2: Ascorbic acid content in fresh fruit by using UV Spectrophotometer.

Conclusions

Spectrophotometric method for determination of vitamin C is simple and reliable method. The fruits which are taken from the local market are good source of vitamin C. The 2,4-dinitrophenylhydrazine spectrophotometric method, with modifications, enabled a rapid determination of the total vitamin C contents of fresh fruit. The preparation of a very clear supernatant is critical for stable and reliable spectrophotometric measurement. For fresh produce such as fruit and vegetables, vacuum micro filtration using cellulose nitrate membrane filter paper Whatman number 0.45 µm or 0.20 µm produced a clear stable supernatant solution for the spectrophotometric estimation of total vitamin C content.

Black current shows maximum amount of vitamin C and grapes shows minimum amount of vitamin C among these samples taken.

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