

Spectrophotometric Determination of Total Vitamin C in Some Fruits and Vegetables at Koya Area – Kurdistan Region/ Iraq

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Abstract

A simple UV- spectrophotometric method for the determination of the total vitamin C (ascorbic acid + dehydroascorbic acid) in various fruits and vegetables at Koya area in Kurdistan Region is described. The spectrophotometric method involves the oxidation of ascorbic acid to dehydroascorbic acid by bromine water in presence of acetic acid. After coupling with 2,4 -dinitrophenyl hydrazine at 37°C temperature for about three hours, the solution is treated with 85% H₂SO₄ to produce a red color complex. Then, the absorbance was spectrophotometrically measured at 280 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.

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. Among the vitamins, vitamin C (ascorbic acid) is an essential micronutrient required for normal metabolic function of the body (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 (Woodall & Ames, 1997). Thus, vitamin C must be obtained through the diet. Vitamin C plays an important role as a component of enzymes involved in the synthesis of collagens and carnitine. Vitamin C is the major water – soluble antioxidant within the body (Sies & Wilhm, 1995; Levine et al., 1986 and Levine et al., 1995). It lowers blood pressure and cholesterol level (Rath, 1993). Not only does a vitamin C intake markedly reduce the severity of a cold, it also effectively prevents secondary viral or bacterial complications. Numerous analysis have shown that an adequate intake of vitamin C is effective in lowering

the risk of developing breast cancer, cervix, colon, rectum, lung, mouth, prostate and stomach(Levine et al.,1996; Block, 1992; Feriet al., 1994; Block, 1991 and Jacobs, 1993). This vitamin is especially plentiful in fresh fruit, in particular citrus fruit, and vegetables (Bendich, 1997).

A lack of vitamin C in the diet causes the deficiency disease scurvy (Levine, 1986). This potentially fatal disease can be prevented with as little as 10 mg vitamin C per day (Weber et al., 1996), an amount easily obtained through consumption of fresh fruit and vegetables. Other symptoms of its deficiency have been reported, but they are not well defined. It participates in numerous biochemical reactions, suggesting that vitamin C is important for every body process from bone formation to scar tissue repair (Grrof et al., 1995). 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 (Lehinger, 1993). Keeping in view its importance; the estimation of vitamin C containing this vitamin assumes significance. A wide variety of food exists that contains vitamin C. It is widely known by the laypeople today that the best sources of vitamin C are citrus fruits and their juices. For better utilization of fruits and vegetables as a human food, clear understanding of their nutrition value as well the content of vitamin C estimation is essential(Rahman et al., 2005). Vitamin C levels in fruits vary considerably due to factors, which include species, maturity, portion, soil, climate, season, handling, method of preparation and consumption (Alam, 1996). There are many citrus fruits and vegetables available at Koya area such as Orange, Tangerine, Sour Orange, Lemon, Green Pepper, Parsley and Tomato, total vitamin C content of these fruits and vegetables are not known. In this study the results of different samples are described and so as to evaluate them regarding the role of in human health and nutrition.

Materials and Methods

An experiment was conducted at Chemistry Laboratory of Koya Technical Institute – Kurdistan Region - Iraq. There are many methods that are employed for the quantitative determination of vitamin C such as biological, electrochemical and chromatographic method (Veasey& Nieman,1980). All the methods have great limitation in use for different purpose, such as in biological sample, food products, pharmaceuticals etc. 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 samples type have its own specific characteristics and properties in terms of extraction, purification, interference of other compounds (such as color, presence of oxidizing, reducing components etc). Although some methods are available for the determination of ascorbic acid but very few methods are employed for the determination of both forms (ascorbic acid and oxidized form, dehydroascorbic acid) of ascorbic acid. This is because of the two forms of the vitamin C: ascorbic acid and its oxidized form dehydroascorbic acid, and the different chemical, optical and electrochemical properties as well.

On the basis of reducing property of ascorbic acid, it can be determined chemically by titrating against an oxidizing agent such as 2,6-dichlorophenolindole dye (Indian Pharmacopoeia). It is not applicable to many pharmaceutical preparations containing Fe (II), Sn(II), Cu(I), SO₂, SO₃⁻², S₂O₃⁻² ions which are usually associated with mineral or liver preparations. The method is applicable only when the concentration of dehydroascorbic acid is negligible. The applicability of the method is restricted to only those samples of citrus fruits and multivitamin tablets, which do not contain minerals. To determine the content of total vitamin C in food samples, a well - established method is the 2, 4-dinitrophenyl hydrazine (DNPH) (Riemschneider et al., 1976). This is a simplified method for the simultaneous determination of the total vitamin C employed coupling reaction of 2,4- Dinitrophenyl hydrazine dye with Vitamin C and followed by spectrophotometric determination.

Instrument

A Thermo Electronic Cooperation spectrophotometer (Model GESEYS 10uv) with 1 cm cell was used.

Reagents required

- 1- 10% Acetic acid
- 2- 10% ThioUrea
- 3- 2,4- Dinitrophenyl Hydrazine
- 4- 85% Sulphuric acid
- 5- Bromine water

Standard vitamin C (ascorbic acid) solution

0.05g standard crystalline ascorbic acid was dissolved in 100ml distilled water to prepare 500ppm standard stock solution.

Sample preparation

Ten grams of the sample was blended or 10g blended sample was homogenized with about 50ml of acetic acid solution. Then it was quantitatively transferred into a 100ml volumetric flask and was shaken gently until a homogenous dispersion was obtained. Then it was diluted up to the mark by 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: To the filtrated sample solution a few drops of bromine water were added until the solution became colored (to confirm the completion of the oxidation of ascorbic acid to dehydroascorbic acid). Then a few drops of thiourea solution were added to it to remove the excess bromine and thus the clear solution was obtained. Then 2, 4-Dinitrophenyl hydrazine solution was added thoroughly with all standards and also with the oxidized ascorbic acid. Total vitamin C employing coupling reaction of 2, 4-Dinitrophenyl hydrazine dye with vitamin C and followed by spectrophotometric determination.

Results and Discussion

Calibration curve: After determining λ_{\max} of the colored complex (280nm) the absorbance of all standards (converted to colored complex) was taken to construct a calibration curve. The calibration curve was constructed by plotting the concentration versus the corresponding absorbance. Molar absorptivity was found $0.0323 \text{ L mol}^{-1}\text{cm}^{-1}$ using Beer – Lambert plots (Fig. 1).

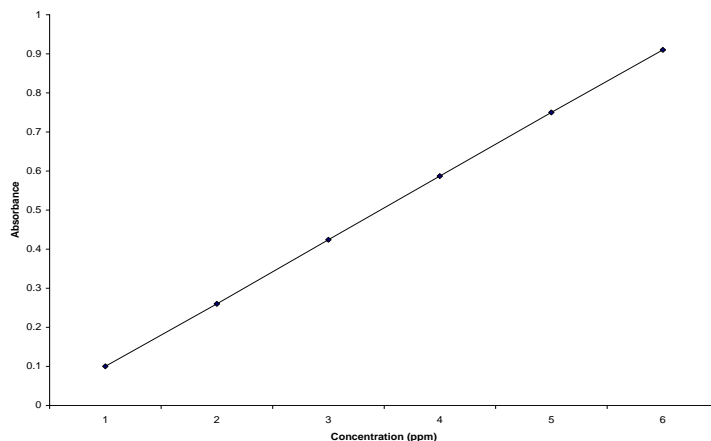


Fig. 1: Calibration curve of standard vitamin C at 280 nm

Determination of vitamin C in sample

Generally all the parts of a fruit and all fruits have no equal amounts of edible part, in the comparative study of 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, also have relatively high amount (7 to 163 mg/100g) of vitamin C (Lidijia et al., 2003; Alam 1996). In this study the locally available fruits such as orange, Tangerine, Sour Orange and Lemon collected from Koya area and the vitamin C content of these fruits were determined. Lemon contain 5.315 mg/10g and orange contain 4.337 mg/10g of total vitamin C. By the similar methods vitamin C content of the Green pepper and Tomato as local vegetables is also determined (Table 1). 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 0.29 to 1.98 % (Table 1). The reliability of this method is also confirmed from the consideration of the following expected interferences.

Table 1: The total vitamin C content in Fruits and Vegetables

Fruits and vegetables	Condition	Total Vitamin C (mg /10g)	SD%
Orange	Fresh	4.337	1.70
Tangerine	Fresh	2.808	1.03
Sour Orange	Fresh	1.868	1.04
Lemon	Fresh	5.315	1.90
Green Pepper	Fresh	1.557	0.56
Tomato	Fresh	0.841	0.29
Strawberry	Fresh	51.74	0.26
Cabbage	Fresh	17.416	0.06
Parsley	Fresh	4.227	0.07

Interferences due to diketogulonic acid

Due to the destructive oxidation hydrolysis at higher PH, this results in the opening of the lactone ring of the ascorbic acid and loose the vitamin activity. These processes naturally occur in fruits, and some amounts of diketogulonic acid is present in the fruits (Geigertj et al., 1981). 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% H₂SO₄. 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 of ascorbic acid. The spectrum shows that there is no considerable absorption peak near the 280nm (the absorption maxima of DNPH complex of ascorbic acid).

Interference due to extracted glucose

As ascorbic acid is largely similar to the glucose by structure, some of glucose may be extracted in the acetic 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 given in Fig. 2. From spectrum it is evident that there is no absorption peak around the interested peak at 280 nm. Vitamin C is important to human health, and many species need a dietary source to stay healthy. The locally available citrus fruits such Orange, Tangerine, Sour Orange and Lemon are the excellent sources of vitamin C. The locally available vegetables which were analyzed contain relatively good amount of vitamin C and a good source of vitamin C. The method is simple and offers an excellent method for the determination of total vitamin C in fruits and vegetables.

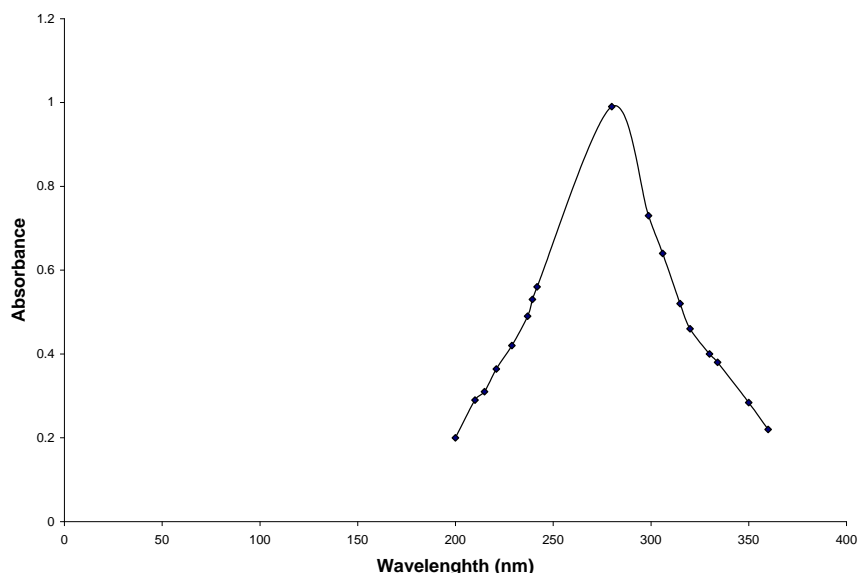


Fig. 2: Spectrum of determination of λ_{\max} of standards of vitamin C

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تعيين كمية فيتامين C الكلية طيفياً في فواكه وخضراوات مختلفة منتجة في منطقة كوية – اقليم كردستان – العراق

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الخلاصة

يقدم هذا البحث وصفا لطريقة طيفية لتحديد الكمية الكلية لفيتامين C (Total Vitamin C) (ascorbic acid + dehydroascorbic acid) في انواع مختلفة من الفواكه والخضراوات المنتجة في مدينة (كوية) محافظة اربيل – اقليم كردستان العراق . تقوم هذه الطريقة على اكسدة ascorbic acid الى dehydroascorbic acid باستخدام ماء البيروم بوجود حامض الخليك. وبعد مفاعلة المحلول مع 2,4-dinitrophenyl hydrazine بدرجة 37°C لمدة ثلاث ساعات، يتم معالجته بمحلول H_2SO_4 85% لتكوين معقد احمر اللون. وبعد ذلك، يتم قياس الامتصاصية عند 280 nm، وقد تبين ان محتوى فيتامين في الفواكه 1.868 – 51.74 mg/10g وفي الخضراوات كان 0.841 – 17.416 mg/10g، إضافة لذلك فقد تم حساب الانحراف المعياري ومناقشة والتداخلات المحتملة.