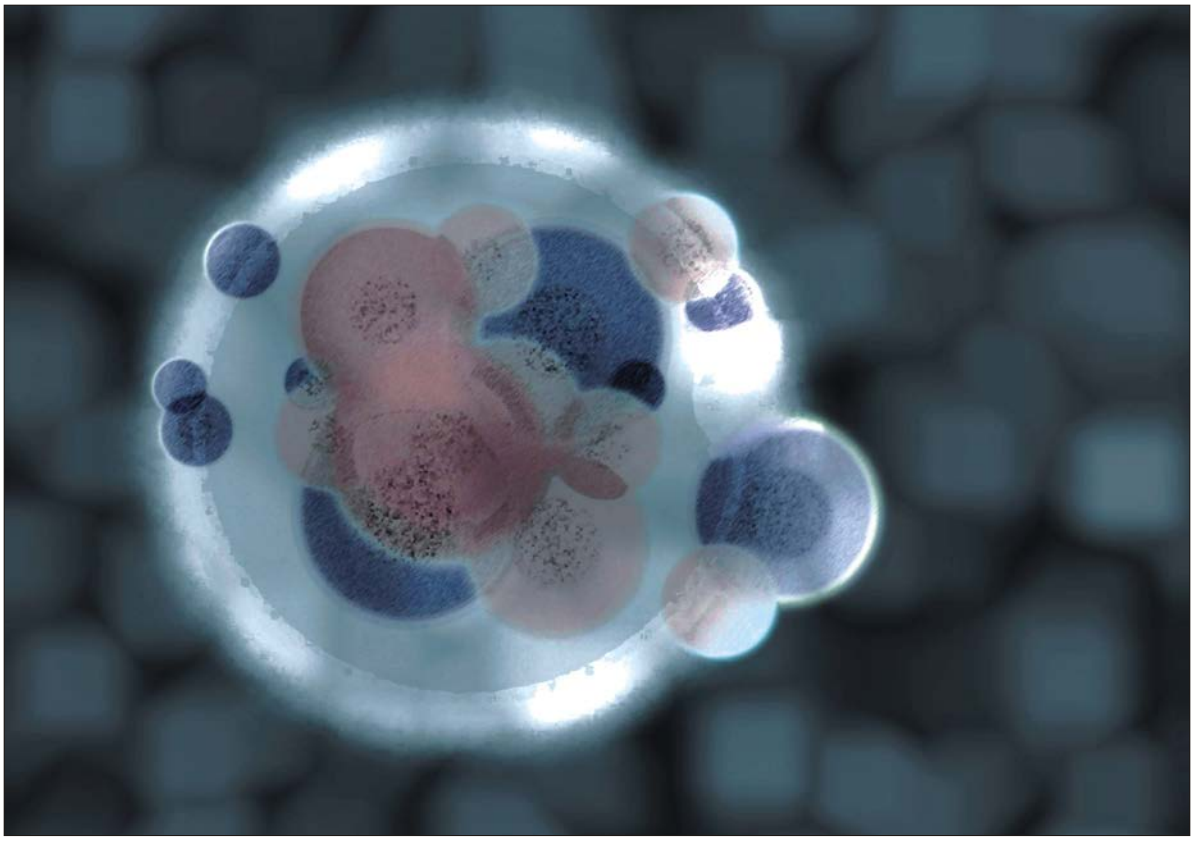

Journal of Biological Sciences

ISSN 1727-3048

science alert

ANSInet
an open access publisher
http://ansinet.com

Journal of Biological Sciences 6 (2): 388-392, 2006
ISSN 1727-3048

A Simple UV-spectrophotometric Method for the Determination of Vitamin C Content in Various Fruits and Vegetables at Sylhet Area in Bangladesh

M.M. Rahman Khan, M.M. Rahman, M.S. Islam and S.A. Begum
Department of Chemistry, Shah Jalal University of Science and Technology,
Sylhet-3114, Bangladesh

Abstract: A simple UV-spectrophotometric method for the determination of the total vitamin C (ascorbic acid + dehydroascorbic acid) in various fruits and vegetables at Sylhet area 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 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 12 to 118 mg/100 g in fruits and 22 to 135 mg/100 g in vegetables. The percent recovery, statistical calculation and the possible interfering factors are also discussed.

Key words: Fruits, vegetables, vitamin C, UV-spectrophotometer, Sylhet

INTRODUCTION

Human health is very important to our survival. Vitamins help the human to maintain a healthy diet. They are serve as essential components of the specific coenzymes and enzymes 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). Humans 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 and 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 and Wilhlm, 1995; Levine *et al*., 1986; Levine *et al*., 1995). It lowers blood pressure and cholesterol levels (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. 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 *et al*., 1996; Block, 1992; Frei *et al*., 1994; Block G., 1991; Jacobs, 1993). The 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/d (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 general people today that the best sources of vitamin C are citrus fruits and their juices. Bangladesh is rich in fruits and vegetables. 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 Bangladesh and various types of special fruits are also available in greater sylhet area. Vitamin C levels in fruits vary considerably due to the factors, which include species, maturity, portion, soil, climate, season, handling, method of

Corresponding Author: Dr. Md. Mizanur Rahman, Associate Professor, Department of Chemistry, Shah Jalal University of Science and Technology, Sylhet-3114, Bangladesh

preparation and consumption. The content of vitamin C in fruits and vegetables found in Sylhet area may be different from others because the properties of soil and climate of Sylhet are totally different from that of the rest part of Bangladesh. These fruits may contain high content of vitamin C. Among the fruits and vegetables are available in Sylhet area, vitamin C content of some fruits and vegetables such as Shatkara, Adalemon are known (Alam, 1996). There are many citrus fruits and vegetables available at sylhet area such as Dumur, Katajamir, Katadanga etc which commonly used locally to make pickle and vitamin C content of these fruits and vegetables are not known. Recently we have reported the estimation of vitamin C content of locally available some citrous fruits (Rahman *et al*., 2005) by 2,4-DNPH method. As a part of our ongoing study on vitamin C estimation we have collected more citrous fruits and vegetables from Sylhet area and estimate their vitamin C content. This study we would like to describe the results of these samples and thus enable us to evaluate them regarding its role in human health and nutrition.

MATERIALS AND METHODS

An experiment was conducted at Organic Chemistry Laboratory of Shahjalal University of Science and Technology University, Sylhet, Bangladesh during March to October, 2005.

There are many methods that are employed for the quantitative determination of vitamin C such as biological, electrochemical, and chromatographic method (East and Nascimeet, 2002; Geigertj *et al*., 1981; Veasey and Nieman, 1980). All the method has 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 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 two forms of the vitamin C, ascorbic acid and its oxidized form dehydroascorbic acid possess the different chemical, optical and electrochemical properties.

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), SO_2, SO_3^{2-} and $S_2O_3^{2-}$ 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 citrous fruits and multivitamin tables, which do not contain minerals.

To determine content of total vitamin C in food samples, a well-established method is the 2,4-dinitrophenyl hydrazine methods (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-dinitrophenylhydrazine dye with vitamin C and followed by spectrophotometric determination.

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

Reagent required

5% Metaphosphoric acid-10% acetic acid: Fifteen grams of solid metaphosphoric acid (E. Merck) were dissolved in 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-Dinitrophenyl-hydrazine solution; 85% Sulphuric acid:

Standard vitamin C (ascorbic acid) solution: 0.05 g standard crystalline ascorbic acid was dissolved in 100 mL of distilled water to prepare 500 ppm standard stock solution.

Sample preparation: Ten grams of sample was blended or 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 acid-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: To the filtered sample solution 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 few drops of thiourea was added to it to remove the excess

Table 1: The total vitamin-C content in fruits and vegetables

Fruits name	Botanical name	% of edible part	Condition			Total vitamin-C (mg/100 g)	Percent of standard deviation (% S)
Dumur	*Ficus hispida*	~98	green	normal		12.20±0.127	1.041
				Deep	freeze		
				storage	7 days	10.55±0.166	1.573
				Deep	freeze		
				storage	14 days	8.09±0.147	1.817
				Deep	freeze		
				storage	21 days	6.34±0.132	2.081
			Ripen	Normal		15.23±0.120	0.788
				Deep	freeze		
				storage	7 days	13.75±0.337	2.451
				Deep	freeze		
				storage	14 days	11.70±0.169	1.444
				Deep	freeze		
				storage	21 days	9.88±0.176	1.781
Aurboroi	Phyllalthus acidus	62-65	Normal			21.75±0.213	0.979
Belumbo	Averrhoa bilimbi	~98	Normal			19.59±0.074	0.377
Peyara	Psidium guajava	100	Mixed (inside and outside)			91.21±0.930	1.020
			Inside			84.68±0.704	0.831
			Outside			188.25±0.187	0.158
Katajamir (lemon)		95-98	Normal			35.83±0.204	0.569
			Deep freeze storage 7 days			32.14±0.410	1.276
			Deep freeze storage 14 days			28.19±0.653	2.316
			Deep freeze storage 21 days			25.77±0.287	1.114
			Deep freeze storage 30 days			22.97±0.465	2.012
			Deep freeze storage 60 days			21.79±0.167	0.766
Katabadam		60-65	Normal			76.62±1.292	1.686
Sajnaleaves	Moringa oleifera lumk	100	Normal			134.70±2.720	2.029
Katadenga		100	Normal			22.85±0.347	1.519
Rojoterpata		100	Normal			58.46±0.946	1.618
			Deep freeze storage 7 days			50.48±0.545	1.08
Thankunipata	Centella asiatica	80-90	Normal			21.70±0.308	1.419
Kalmishak	Ipomoea aquatica forsk	100	Normal			29.10±0.376	1.292

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.

Reactions

- Ascorbic acid is oxidized to dehydroascorbic acid by the action of bromine solution.
- 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 than 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 λ_{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 0.0323 L mol^{-1} cm^{-1} using Beer-Lambert plots (Fig. 1).

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 to 163 mg/100 g) vitamin C (Lidija *et al.*, 2003; Alam 1996). In study the locally available fruits such as Dumur, Katabadam, Katadenga etc. collected from Sylhet and the vitamin C content of these fruits were determined. Dumur is not popular fruit but contain 15 mg/100 g and Katabadam contain 77 mg/100 g of vitamin C. By the similar methods vitamin C content of the fruits Arboroi, Peyera (Guava), Katajamir and

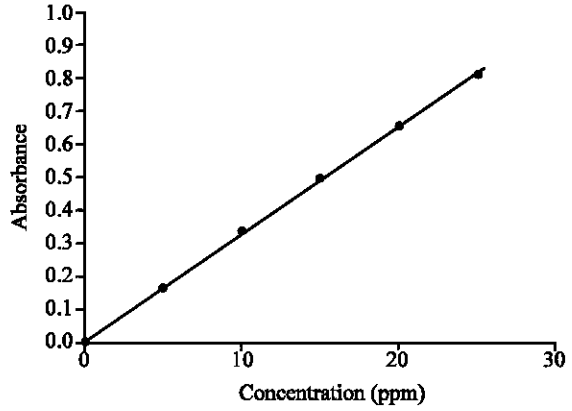

Fig. 1: Calibration curve of standard vitamin C at 521 nm

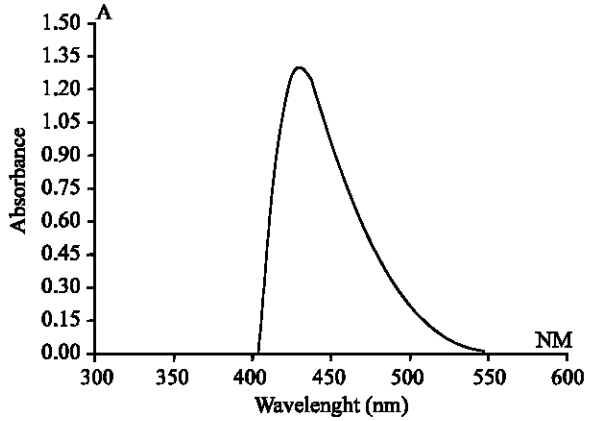

Fig. 2: Spectrum of DNPH complex of glucose

vegetables such as Rojottepata, Thankoni, Kalmishak etc is also determined. Since the vitamin C content vary with the storage period on preservation, therefore, Vitamin C content of some fruits and vegetables were also determined at frozen conditions. It is observed that vitamin C content of fruits and vegetables slowly decreases with time as the storage period increase even at low temperature (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 0.20 to 2.45 % (Table 1). The reliability of this method is also confirmed from the consideration of the following expected interferences.

Interferences due to diketogulonic acid: Due to the destructive oxidation hydrolysis at higher pH results the opening of the 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 (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% 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).

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

Vitamin C is important to human health, and many species need a dietary source to stay healthy. The locally available citrous fruits such Katagamir, Katabadam and Peyar are the excellent sources of vitamin C. The locally available vegetables which was analyzed contain relatively good amount of vitamic 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.

ACKNOWLEDGMENTS

We are grateful to the Department of Chemistry, Shah Jalal University of Science and Technology, Sylhet-3114, Bangladesh for giving necessary facilities during research.

REFERENCES

Alam , M.A., 1996. Comparative study of total vitamin C in various fruits and vegetables of greater Sylhet area, M.Sc. Thesis, SUST, Sylhet.

Bendich, A., 1997. Vitamin C safety in humans. In: Packer, L. and J. Fuchs (Eds.). Vitamin C in health and disease. New York: Marcel Dekker Inc., pp: 367-79.

Block, G., 1991. Epidemiologic evidence regarding vitamin C and cancer. Am. J. Clin. Nut., 54: 1310S-1314S

Block, G., 1992. The data support a role for antioxidants in reducing cancer risk. Nut. Rev., pp: 207-213

Frei, B., 1994. Reactive oxygen species and antioxidant vitamins: mechansims of action. Am. J. Med., 97: Suppl. 3A, 5S-13S

Grrof, J.L., S.S. Gropper and S.M. Hunt, 1995, The water soluble vitamins.In: Advanced nutrition and human metabolisms: West publishing company, pp: 222-237.

Geigertj J., D.S. Hirano and S.L Neidleman, 1981. High-performance liquid chromatographic method for the determination of L-ascorbic acid and D-isoascorbic acid. J. Chromatograp., 206: 396-39.

Indian Pharmacopoeia, 1985. Photolithio Press, Faridabad, pp: 49.

Jacobs, M.M., 1993. Diet, nutrition, and cancer research: An overview, Nutrition Today, pp: 19-23.

Jaffe, G.M., 1984. Vitamin C, In: Machalinal ed. Handbook of vitamins. New York: Mercell Dekker Inc., 199-244.

Lidija B., Pfendt, L. Vesna L. Vukasinovic, Nada Z. Blagojevic and Mirjana P. Radojevic, 2003. Second order derivative spectrophotometric method for determination of vitamin C content in fruits, vegetables and fruit juices. Eur. Food Res. Technol., 217: 269-272.

Lehinger Albert L. 1993. Principles of Biochemistry, CBS Publishers and Distribution Pvt. Ltd.

Levine, M., 1986. New concepts in the biology and biochemisty of ascorbic acid. New England. J. Med., 314: 892- 902 .

Levine, M. *et al*., 1995. Determination of optimal vitamin C requirements in humans. Am. J. Clin. Nut., 62: 1347.

Levine, M. *et al*., 1996. Vitamin C pharmacokinetics in healthy volunteers: Evidence for a recommended dietary allowance. Proceedings of the National Academy of Sciences USA, 93: 3704-3709.

Levine, M., 1986. New concepts in the biology and biochemistry of ascorbic acid. N. Engl. J. Med., 314: 892-902.

Rath, M., 1993. Eradicating Heart Disease. Health Now, San Francisco, CA.

Riemschneider, R., M.Z. Abedin and R.P. Mocellin, 1976, Qualitats and stabilisierungprufung hitzekonservierter Nahrungsmittel unter verwendung von Vit C als kriterium-Mitt 1. Alimenta, 15: 171.

Khan, M.M., M.M. Rahman, A.T.M. Murad and S.A. Begum, 2005. Determination of vitamin C content in various fruits and vegetables by UV-spectrophotometric method at Sylhet area, Bangladesh. J. Environ. Sci., 11: 190-193

Sies, H. and S. Wilhelm, 1995. Vitamins E and C, Beta-carotene, and other carotenoids as antioxidants. Am. J. Clin. Nutr., 62: 1 315S-321S.

Veasey, R.L. and T.A. Nieman, 1980. Chemiluminescence high-performance liquid chromatographic detector applied to ascorbic acid determinations. J. Chromatogr., 200: 153-162.

Woodall, AA. and BN. Ames, 1997. Diet and oxidative damage to DNA: The Importance of Ascorbate as an Antioxidant. In: Packer L. and J. Fuchs, Eds. Vitamin C in health and disease. New York: Marcel Dekker Inc, pp: 193-203.

Weber P, A. Bendich and W. Schalch, 1996. Vitamin C and human health-a review of recent data relevant to human requirements. Intl. J. Vitam. Nutr. Res., 66: 19-30.