SPECTROPHOTOMETRIC DETERMINATION OF TOTAL VITAMIN C CONTENT IN DIFFERENT FRUITS AND VEGETABLES CONSUMED IN TANSEN, PALPA

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Abstract

Vitamin C plays an important role in metabolism and is found in many fruits and vegetables. The purpose of this study was to estimate the total vitamin C content of some fruits and vegetables available in Tansen, Palpa, Nepal using the spectrophotometric method. In this method, ascorbic acid was oxidised to dehydroascorbic acidat 370C with bromine water in a solution of acetic acid, which was coupled with 2,4-dinitrophenylhydrazine (DNPH). This solution produced a red-coloured complex with 85% H2SO4. The absorbance of that complex was determined at 530 nm.Fifteen samples of fruits and egetables (lemon, grapes, orange, tomato, banana, pomegranate, sugarcane, carrot, apple, cucumber, potato, cauliflower, cabbage, sweetlime, and pomelo) were collected from the local market of Tansen to determine vitamin C content. Among fruits, vitamin C content was maximum in lemon (76.122 ppm) and minimum in cucumber (17.90 ppm), whereas, in vegetables, it was maximum in potato (26.107 ppm) and minimum in cabbage (13.106 ppm). The values of vitamin C content in different samples of fruits and vegetables were compared with the available literature values. This study has contributed to understanding the concentration of vitamin C in various fruits and vegetables.

Keywords: Fruits and *Vegetables*, Metabolism,Scurvy, Spectrophotometer, Vitamin C

Introduction

Vitamins are non-energy producing organic compounds essential for growth and nutrition. Vitamins perform different functions in the body. They are required in small quantities in the diet. A healthy diet may provide most people with all of the vitamins they require. Vitamin deficiencies are caused by insufficient dietary intake, malabsorption, increased excretion, increased tissue demands, and genetic disorders. (Desai & Desai, 2019).They play a key role in the metabolism and other specialized functions of numerous coenzymes and enzymes (Rahman et al., 2007).

One of the most vital vitamins for human health is vitamin C(Rahman et al., 2007).It is a six-carbon organic acid with a glucose-like structure, having the molecular formula $C_6H_8O_6$anda molar mass of 176.13 g/mol (Elhefian et al., 2019). Vitamin C is found in nature primarily as isomer L.The isomer D can also be found. However, it has little biological activity (Zanini et al., 2018). The daily requirement for vitamin C is 100-200 mg(Reddy & Naga, 2017).

"Vitamin C is required for the formation of gums, arteries, soft tissues, and bone (collagen synthesis), brain and nerve function (neurotransmitter and hormone synthesis), nutrient metabolism (primarily iron, protein, and fat), and antioxidant defence (directly and through

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vitamin E reactivation) against free radicals (free radicals increase the risk of cancer and cardiovascular disease"(Levine et al., 1995; Rahman et al., 2007). It helps to regulate blood pressure, reduce the intensity of colds, and guard against subsequent viral or bacterial problems. Different types of cancers are reduced by enough vitamin C intake. (Khan et al., 2006; Levine et al., 1995; Rahman et al., 2007).Scurvy is caused by a lack of vitamin C and causes bleeding, gum disease, tooth loss, poor tissue development, and wound healing. Excessive consumption can induce stomach and intestinal irritation, kidney stones, and copper deficiency. The different types of food materials contain vitamin C. The amounts of vitamin C in various fruits and vegetables vary. Citrus fruits and their juices are the best sources of vitamin C. There may be a significant loss of vitamin C in fruits and vegetables if they are ripened, cut, cooked, washed, thermic processed, and exposed to atmospheric oxygen (Shara & Mussa, 2019). It is recommended, however, that these edibles be consumed fresh.

(Shrestha et al., 2016) investigated the levels of ascorbic acid in different citrus fruits collected from Kathmandu valley, Nepal. The average concentration of pomelo was 61.29mg/100ml and the citron was about 17.4mg/100ml.(Majidi & Al-Gubury, 2016) used two methods to determine ascorbic acid in various fruits and vegetables. The spectroscopy method was superior to the titrimetric method in determining the value of ascorbic acid. According to the researchers, oranges and kiwis had very high levels of ascorbic acid. At the same time, it was very low in red pepper, cabbage, and tomatoes.(Desai & Desai, 2019)used a simple and reliable spectrophotometric method to determine the vitamin C content in different fruits in the South Gujarat Region, India. They found that blackcurrants contained the highest amounts of vitamin C, whereas grapes had the lowest. (Elhefian et al., 2019)reported a wide range of vitamin C levels in common fruits and vegetables in north-western Libya. Guava and green pepper were shown to be the most vitamin C-rich fruits and vegetables, respectively. (Sharma et al., 2019) studied titrimetric and spectroscopic methods to determine ascorbic acid in fresh and marketed fruits. Lemons and oranges contained the most vitamin C, whereas apples and grapes had the least. They concluded that the titration method is simple, UV-spectroscopy is less time-consuming and easy to interpret.

People in Tansen consume different types of fruits and vegetables obtained from their local area,various parts of the country, or even from India. For better utilisation of fruits and vegetables, it is essential to know their nutritional value, vitamin C content, and factors affecting their content. Different factors, like species, time of maturation, climate, season, nature of the soil, method of handling, preparation, and consumption, affect the vitamin C content (Rahman et al., 2007).Different fruits and vegetables are usually available in Tansen.The fruits and vegetables included in this study were apples, oranges, grapes, bananas, lemons, sugarcane, pomelo, pomegranates, carrots, cucumbers, potatoes, sweet limes, cabbage, tomatoes, cauliflower.It is unknown how much vitamin C these fruits and vegetables contain. In this study, total vitamin C content in various fruits and vegetables consumed in Tansen, Palpa, was determined using the spectrophotometric method. The obtained results were compared with the literature values available. Therefore, the determination of vitamin C is significant in light of its importance.

Materials and Methods

From September 2019 to April 2020, an experiment was performed in the laboratory of the Department of Chemistry, Tribhuvan Multiple Campus, Tansen, Palpa.In this study, an oxidative method of determining vitamin C was used, which involved oxidizing ascorbic acid to dehydroascorbic acid in the presence of bromine water and acetic acid to determine total vitamin C (Kapur et al., 2012; Rahman et al., 2007).A red colour complex was produced after three hours of coupling with 2,4-dinitrophenyl hydrazine at 37°C using 85 per cent H_2SO_4, and the absorbance was measured at 530 nm(Kapur et al., 2012; Rahman et al., 2007).The DNPH method is the most accurate, simplest, and widely applicable method of determining the total ascorbic acid content of fresh foods, including fruits and vegetables(Kapur et al., 2012; Rahman et al., 2007).

Instruments

Scanning visible spectrophotometer Model El-2306 with 1 cm cell

Electronic analytical weighing balance

Thermostatic water bath (Grant OLS-200)

Chemicals and Reagents used

All the chemicals were laboratory grade and used without further purification.

3% Metaphosphoric acid-8% glacial acetic acid

A mixture of 40 ml of acetic acid and 450 ml of distilled water was used to dissolve 15 grams of solid metaphosphoric acid in a 500 ml volumetric flask. The solution was then collected after it was filtered.

10% Thiourea solution

In a 100 ml volumetric flask, 10 g of thiourea was dissolved in distilled water, agitated, and the volume was filled to the mark.

2, 4-dinitrophenylhydrazine solution

3 grams of 2, 4-dinitrophenylhydrazine was mixed with 20 ml of water and 70 ml of 95 percent ethyl alcohol in a flask.The flask was placed in an ice bath in a beaker and stirred rapidly until it cooled to 10°C.Then 15 ml of conc.H_2SO_4 was slowly added while the mixture was being stirred continuously. Once the temperature reached 20°C, H_2SO_4 was added and stirred until the temperature dropped back to 10°C before being removed from the ice bath and placed on a stirrer-hotplate.After dissolving 4 DNPH, or when 60°C was reached, the flask was stirred without heating, and the cooled solution was filtered through a fritted funnel. The filtrate was transferred to the volumetric flask.

85% Sulphuric Acid

85 ml of conc. H_2SO_4 was diluted and made up to 100 ml in the volumetric flask by adding 3

15 ml of distilled water.

Standard vitamin C (ascorbic acid) solution

The ascorbic acid (0.05 g) was dissolved in distilled water and diluted to 100 ml in a volumetric flask to prepare a standard stock solution containing 500 ppm of ascorbic acid.A stock solution of ascorbic acid (500 ppm) was diluted to make five standard solutions (5, 10, 15, 20, and 25 ppm)as shown in Table 1.

Table 1: *Preparation of standard ascorbic acid*

Ascorbic acid Solution	2,4 DNPH	Bromine water and Thiourea	Metaphosphoric-acetic acid	85% sulphuric acid
1ml (5ppm)	1 ml	1 ml	92 ml	5 ml
2ml (10ppm)	1 ml	1 ml	91 ml	5 ml
3ml (15ppm)	1 ml	1 ml	90 ml	5 ml
4ml (20ppm)	1 ml	1 ml	89 ml	5 ml
5ml (25ppm)	1 ml	1 ml	88 ml	5 ml

Sample preparation

Each sample was cut into small pieces, blended with an electric blender.50 ml of 3% metaphosphoric acid and 8% acetic acid solutions were mixed with 10 ml of the blended sample solution to homogenize it. To dilute the mixture to the desired concentration,8 % acetic acid and 3 % metaphosphoric acid solution was added to a 100 ml volumetric flask. A clear filtrate solution was obtained by filtering the obtained solution and determining its vitamin C content.

Determination of vitamin C

A few drops of bromine water were added to the filtered sample solution to oxidize the ascorbic acid to dehydroascorbic acid. The excess bromine was then removed with 10% Thiourea, resulting in a clear solution.Standard solutions of ascorbic acid were prepared by diluting a 500-ppm stock solution into solutions of 5, 10, 15, 20, and 25 ppm.One ml of 2-4 DNPH solution with oxidised ascorbic acid and all standards was added thoroughly. All samples and standards were kept in a 37°C water bath for 3 hours to complete the reaction. The samples were then cooled in an ice bath for 30 minutes, followed by constant mixing with 5 ml of 85% H_2SO_4.It resulted in a coloured solution with absorbance at 530 nm.

Reactions

The addition of bromine oxidized ascorbic acid into dehydroascorbic acid.Then, it reacted with 2,4-DNPH to produce an osazone.It became a light red solution when treated with 85% concentrated H_2SO_4. The content of ascorbic acid in the analysed samples was determined using a standard calibration plot (Rahman et al., 2007).

Result and Discussion

Calibration curve

Standard solutions of ascorbic acid at a concentration of 15 ppm were used to determine the absorption maximum. The absorbance was measured in the wavelength range of 500-550 nm using the procedure. The absorption maximum was found at 530 nm.Using all standards and the wavelength of maximum absorbance(λmax) of a coloured complex (530 nm), the calibration curve was constructed. In the 5-25 ppm range, the plot was linear and followed Beer-Lambert's law. The calibration curve was used to calculate the quantity of total vitamin C contained in the sample of fruits and vegetables.

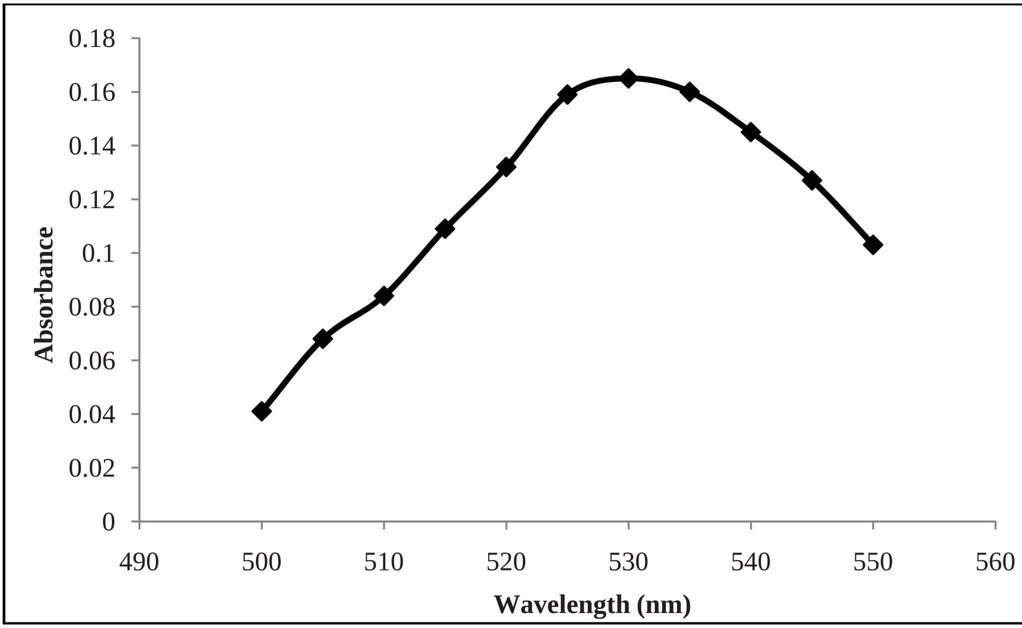

Figure 1. *Absorption maximum*

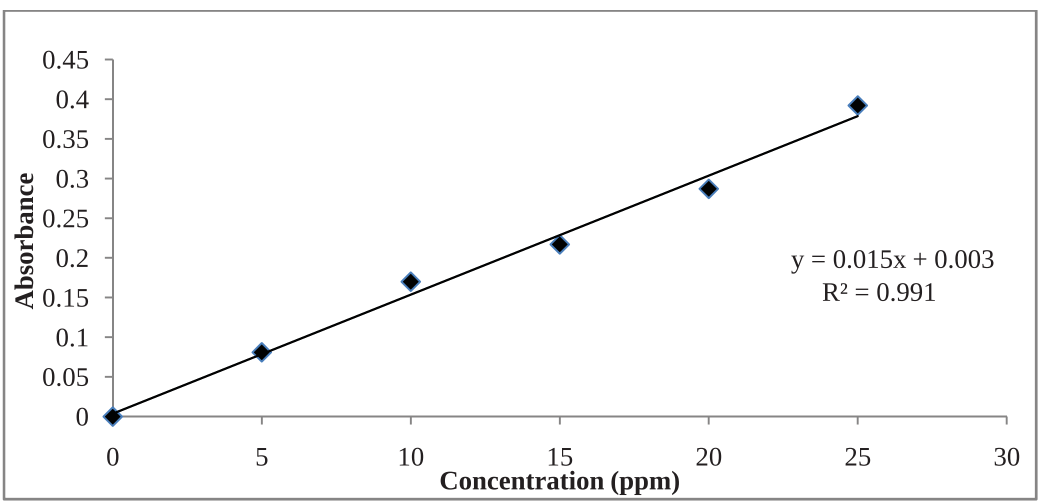

Figure 2: *Calibration curve for standard ascorbic acid at 530 nm*

Determination of Vitamin C in samples

Vitamin C concentration was tested by preparing samples of various fruits and vegetables. Table 2 shows the vitamin C content of the samples studied using the spectrophotometric method.

Table 2:*Total vitamin C content in fruits and vegetables*

S.N.	Samples	Absorbance	Concentration(ppm)
1	Lemon	1.154	76.122
2	Grapes	0.59	38.854
3	Orange	0.557	36.673
4	Tomato	0.556	36.607
5	Pomegranate	0.489	32.180
6	Sugarcane	0.379	24.912
7	Carrot	0.361	23.722
8	Banana	0.351	23.061
9	Apple	0.285	18.700
10	Cucumber	0.273	17.907
11	Potato	0.397	26.101
12	Cauliflower	0.395	25.969
13	Cabbage	0.352	23.128
14	Sweat lime	0.763	50.285
15	Pomelo	0.690	45.462

A total of fifteen different types of fresh fruits and vegetables were studied. Table 2 lists the total vitamin C content of fruits and vegetables, with concentration values ranging from 76.122 ppm to 17.907 ppm. The result showed lemon has the maximum vitamin C content (76.122 ppm), and in cucumber, it was a minimum (17.907 ppm) among fruits. It was maximum in potato (26.101 ppm) and minimum in cabbage (23.122ppm) among vegetables. All citrus fruits have higher vitamin C content. The results obtained are comparable to the literature values available.(Kapur et al., 2012; Shrestha et al., 2016; Vasanth Kumar et al., 2013).When comparing vitamin C concentrations in fruits and vegetables, the data revealed a distribution of total ascorbic acid values, with all citrus fruits ranking high in ascorbic acid content.

Conclusion

The 2,4-DNPH spectrophotometric technique for determining vitamin C in fruits and vegetables is straightforward and accurate. A significant amount of vitamin C can be found in the fruits and vegetables that were taken in this study, which are easily available in local markets. These fruits and vegetables are rich in vitamin C. Therefore, when they are consumed in a relatively large amount, they will provide a daily human dietary intake of the vitamin. C.

Acknowledgements

The authors are grateful to the Department of Chemistry, Tribhuvan Multiple Campus, Tansen, Palpa, for providing necessary facilities during research.

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