

UV Spectroscopic Method for Determination of Vitamin C (Ascorbic Acid) Content in Different Fruits in South Gujarat Region



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Submission: July 29, 2019; **Published:** August 21, 2019

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Abstract

In the present study, determination of ascorbic acid content is determined in different fruits by using UV spectroscopy. In this method bromine water is added which oxidizes the ascorbic acid into dehydroascorbic acid. 2,4 dinitrophenyl hydrazine gives coupling reaction at 37°C temperature for 3 hours. After 3 hours solution is treated with 85% H₂SO₄ which gives coloured complex and the absorbance was measured at 491nm. The content of vitamin C was found maximum in Black currant and minimum in Grapes.

Keywords: UV spectrophotometer; South Gujarat region; Ascorbic acid; Fruits

Introduction

Vitamins are organic compound (non-energy producing), which are essential for normal human metabolism that must be supplied in small quantities in the diet. The definition excludes the inorganic essential trace minerals and essential amino acids and fatty acids which are required in much larger quantities. Other substances needed for proper growth of microorganism or cells in culture are called growth factors. The different chemical forms and precursors of vitamin can be called its vitamers. The vitamins as a drug is necessary in the prevention and treatment of deficiency disease. Some vitamins do have major uses in pharmacological uses in pharmacological doses. Vitamin deficiency occur due to inadequate intake, malabsorption, increased tissue needs, increased excretion, certain genetic abnormalities and drug vitamin interaction. Vitamin C is a major water-soluble antioxidant. Generally, vitamin C is 6 carbon organic acid with structural similarity to glucose. It acts as a potent reducing agent and its l form is generally more active. Citrus fruits are rich sources of vitamin C. According to recent research human milk contains more amount of vitamin C than the cow's milk. It is mostly absorbed from g.i.t and widely distributed extra and intra muscularly. Plasma concentration and total body store of vitamin c depends upon daily intake of ascorbic acid content food. The usual 60mg/day intake results in about 0.8mg/dl in plasma and 1.5g in the body as a whole. Increasing proportion are excreted in urine with higher intake. Body is not able to store more than 2.5g. It

is partly oxidised to active (dehydroascorbic acid) and inactive (oxalic acid) metabolites. The therapeutic uses of ascorbic acid include prevention of ascorbic acid deficiency in patient at a risk and in infants, treatment of scurvy, in anaemia, to acidify the urine in urinary tract infection. They lower blood pressure and cholesterol level in the body. Also reduces the cold and has beneficial effect in the bacterial infection. Adequate amount of vitamin c intake may also prevent developing cancer of breast, cervix, colon etc.

We get vitamin C from the food particularly fruit and vegetables. 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 was first isolated in 1928. In 1932 it was proved to be the agent, which prevents scurvy. Scurvy is a diseased condition which occurs due to deficiency of vitamin C in the body. 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. Many analytical methods were used for ascorbic acid determination, including Titrimetric spectrophotometry and Chromatography, titrimetric, voltammetry, fluorometry, potentiometry as an analytical technique. Similarly, liquid chromatography capillary electrophoresis and gas chromatography were also used for the

determination of ascorbic acid from different species of citrus fruits. UV Spectrophotometry is mostly used to determine ascorbic acid because it is simple method, and Vitamin C is able to absorb UV rays. The method is suitable for use with vitamin C tablets, fresh or packaged fruit juices and solid fruits and vegetables.

2,4 DNPH method of determining ascorbic acid content involves coupling reaction. This method is used in determining ascorbic acid content in different fruits and vegetables. 2,4 DNPH act as a dye in this method. In this method the total amount of vitamin C (Ascorbic acid + Dehydroascorbic acid) is determined in fruits by using UV spectrophotometer. Here bromine water is used which oxidizes the ascorbic acid into dehydroascorbic acid in the presence of acetic acid. Then known amount of 2,4 DNPH is added which gives coupling reaction. Solutions are kept for 3 hours. After 3 hours 85% H₂SO₄ is added which gives coloured solution. These solutions are then measured for determining ascorbic acid content by using UV spectrophotometer.

Materials and Methods

Materials: 5% Metaphosphoric acid-10% acetic acid, 10% Thiourea solution, 2,4-Dinitrophenylhydrazine solution, 85% Sulphuric acid

Instrument

- i. UV- Visible spectrophotometer (Double beam) having matched quartz cells of light path 1cm.
- ii. Model: Shimadzu 1800
- iii. Software: UV probe Version of software: 2.42
- iv. Electronic analytical weighing balance (REPTECH)
- v. Volumetric flask (Borosilicate),
- vi. Pipettes,
- vii. Conical flask.

Samples

Star gooseberries, mulberry, karanda, black current, grapes, mango, guava, wax apple, muskmelon, watermelon, pomegranate, lemon (sweet lemon).

Standard ascorbic acid solution

Standard ascorbic acid solution was prepared by dissolving 50mg of AA in 100ml of distilled water. (500µg/ml)

Preparation of calibration curve

Calibration curve of different concentration i.e. 5,10,15,20,25µg/ml was prepared by proper dilution method.

Sample extract preparation

Sample extract is prepared by blending 10g of sample in the blender. Then sample was mixed with 50ml of 5% metaphosphoric acid acetic acid solution and transferred to the 250ml conical flask. Remaining amount of 50ml of phosphoric acid solution was added into the flask. Then the solution was filtered using Whatman filter paper and the filtrate was collected for determination of vitamin C.

Procedure for estimation of vitamin C

To the filtered sample solution few drop of bromine solution was added and mixed. Then few drops of thiourea solution was added into the sample solution to remove excess of the bromine solution.

Then 1ml of 2,4 DNPH solution was added to the sample solution and to all the standard calibration curve (5,10,15,20,25µg/ml). Coupling reaction occurs due to 2,4 DNPH solution. To complete the reaction all the standards and sample solution were kept at 37°C for 3 hours.

After 3 hours solutions were cooled on ice bath and 5ml of H₂SO₄ was added. As a result, coloured solutions were obtained whose absorbance was measured at specific wavelength.

Reaction

- i. Ascorbic acid is oxidized to dehydroascorbic acid by the action of bromine solution.
- ii. L-dehydroascorbic acid reacts with 2,4dinitrophenylhydrazine and produces an osazone which on treatment with H₂SO₄ forms red coloured solution.

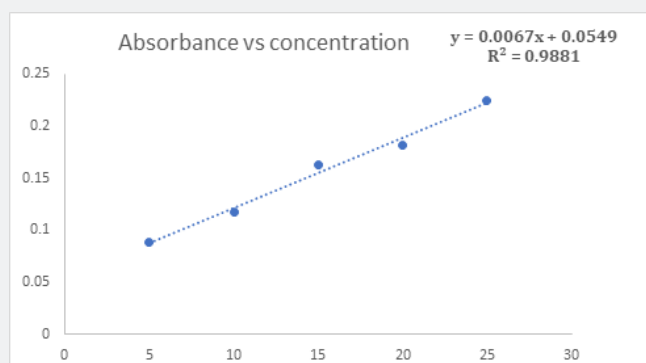


Figure 1: Calibration curve of ascorbic acid.

Result and Discussion

Calibration curve

After determination of λ_{max} of coloured solution the absorbance of all the standards were taken to construct the calibration curve. The calibration curve was constructed by plotting absorbance versus concentration (Figure 1).

Determination of vitamin C using UV-Spectrophotometer

In this work for determination 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 [1-15] (Table 1, Figure 2).

Table 1: Ascorbic acid content in fresh fruit by using UV Spectrophotometer.

Sr No.	Sample	Biological Name	Amount of Vitamin C (mg/100gm)
1	Star gooseberries	Phyllanthus acidus	8.29
2	Mulberry	Morus Nigra	41.8
3	Karanda	Carissa carandas	44.68
4	Black currant	Ribes nigrum	446.83
5	Grapes	Vitis vinifera	5.58
6	Mango	Magnifera indica	36.41
7	Lemon	Citrus limetta	56.4
8	Watermelon	Citrullus lanatas	9.79
9	Muskmelon	Cucumis melo	44.73
10	Wax apple	Syzygium samarangense	18.89
11	Pomegranate	Punica granatum	8.22
12	Guava	Psidium gujava	181.79

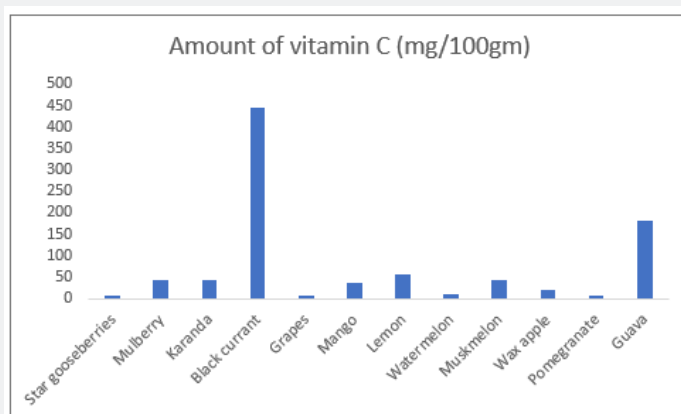


Figure 2: Ascorbic acid content in fresh fruit by using UV Spectrophotometer.

Conclusion

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. This method (2,4 DNPH) is simple and reliable and provide excellent result for determination of vitamin C. Black current shows maximum amount of vitamin C and grapes shows minimum amount of vitamin C among these samples taken.

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DOI: [10.19080/IJESNR.2019.21.556056](https://doi.org/10.19080/IJESNR.2019.21.556056)

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