

Determination of Vitamin C(ascorbic acid) Contents in various fruit and vegetable by UV-spectrophotometry and titration methods

Mohammed Idaan Hassan AL Majidi*, Hazim Y-ALQubury

Babylon University, College of Science, Chemistry Department

*Corresponding author: E-Mail:h.yahya40@yahoo.com

ABSTRACT

Total vitamin C (ascorbic acid + dehydroascorbic acid) has been investigated using two methods, the first UV-spectrophotometric method and the second titration method. In the two methods, a blended sample of various fruit and vegetable in Hill region is homogenized with 85% Sulphuric acid-10% acetic acid solution, the effect of bromine water has been added to oxidize ascorbic acid to dehydroascorbic acid with using acetic acid. In this step the excess of percentage of bromine has been removed when added 3-4 drops of 10% thiourea. After coupling with 2,4-dinitrophenyl hydrazine at 37°C temperature two hours the solution has been cooled by using ice bath after that solution must treated with dilute sulphuric acid to produce a red color complex all prepared solution of fruits and vegetables has been measured at maximum absorbance 280nm. The results revealed that ascorbic acid content was very high in Orange and Kiwi while it was very low in Red pepper, Cabbage and Tomato. The comparison between the two methods the spectroscopy method has been preferred to obtain the value of ascorbic acid than the titrimetric method.

Keyword: Iodimetric titration, UV- spectrophotometric, Ascorbic acid, 2,4-dinitrophenylhydrazine.

1. INTROUDICATION

The scientists prove that the vitamin c have been important vitamin for the human health .Ascorbic acid is reversibly oxidized to form L-dehydroascorbic acid (DHA) which also exhibits biological activity. Dehydroascorbic acid has been converted into acetic acid in the human body. For determine the activity of vitamin c its important determined both acetic acid and Dehydro ascorbic acid in fruits and vegetables. Vitamin C is the real water-soluble antioxidant within the body. It lowers blood pressure and levels cholesterol .Recently many articles has been shown that the effect of vitamin c reduced the risk of developing cancers of breast, colon, rectum, lung, mouth. Vitamin C is generally non- Toxic. Vitamin c very important for everybody such as formation bone and tissue repair. Vitamin C is generally non - toxic. To maintain a good and sound health and the prevention of colds a healthy body, the human must remain saturated with vitamin C. Keeping in view its importance; the estimation of vitamin C containing this vitamin assumes significance. And a wide range of nutrients present contains vitamin C. It is known widely by ordinary people today that the best sources of vitamin C are citrus fruits and juices. To make better use of fruits and vegetables as food, mortal, and a clear understanding of the nutritional value, as well as estimating the content of vitamin C is necessary.

The aim of this project has been estimate the value of ascorbic acid .Ten common Iraqi fruits using methods iodometric titration and UV-spectrophotometry under three temperature regimes representing the ranges the fruits may be exposed to during processing and storage. The redox reaction is preferable to an acid base titration because a number of other species in juice can act as acids. This work helped to demonstrate the effects of processing and storage on the ascorbic acid contents of these fruits.

2. MATERIALS AND METHODS

Materials: 10% Acetic acid, 10% Thio Urea, 2,4- Dinitrophenyl Hydrazine, 85% Sulphuric acid, 5- Bromine water, 10% potassium iodide, 1 M potassium iodate (KIO₃), Sodium thiosulphate, 3% Starch Solution

Instrument: Apple –UV-visible spectrophotometer with 1 cm cell was used.

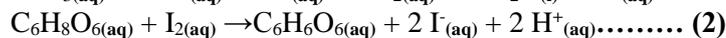
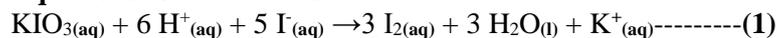
Sample preparation and evaluation of ascorbic acid by method spectrophotometer: In this work different kind of fruits and vegetables has been choice for investigated to determine the amount of ascorbic acid in this samples. All samples has been blended then filtered using Buchner, 10 gm of each sample was transferred into a 100ml volumetric flask homogenized by using 50ml acetic acid solution with shaken, 4-5 drops of bromine water has been added until the solution became colored, 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. Then complete the solution up to the mark with acetic acid. The absorbance for all samples has been measured using Apple –UV-visible spectrophotometer to determine the concentration of ascorbic acid in the fruits and vegetables under testing.

Sample preparation and evaluation of ascorbic acid by method redox Titration: 10 ml of each of the fruit and vegetable samples was pipetted into a pre-washed conical flask and 5 ml of 10% potassium iodide (KI) with 1 ml of 0.3 M sulphuric acid (H₂SO₄) were added into the flask. 10 ml of 0.01 M potassium iodate (KI₃) was also added into the flask. The excess iodine generated was titrated against 0.01 M sodium thiosulphate (Na₂S₂O₃) solution blank titration was carried out with 10 ml of distilled water.

Amount of ascorbic acid obtained from titration method: The amount of Ascorbic Acid is calculated from the expression:

$$(\text{meq KIO}_3 - \text{meq Na}_2\text{S}_2\text{O}_3) = \text{meq A.A}$$

Equations for the reaction:



Ascorbic acid dehydroascorbic acid



Statistical Analysis: The results were expressed as mean \pm standard error of mean of three determinations.

3. RESULTS AND DISCUSSION

Calibration curve: The calibration curve has been carried out using a series of solutions ascorbic acid has been prepared from stock solution of ascorbic acid 500ppm. By plotting the concentration rang (1 – 20 ppm) versus the corresponding absorbance determined the concentration of ascorbic acid in all fresh samples of fruits and vegetables has been choice.

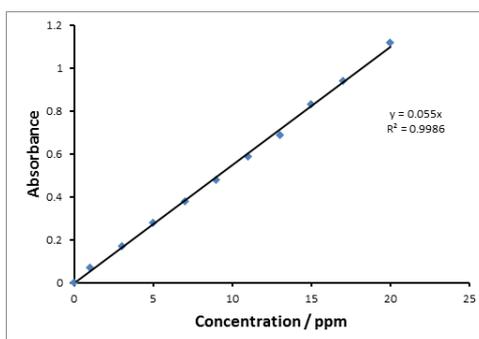


Fig.1. Calibration curve of standard ascorbic acid at 280 nm

Determination of vitamin C using UV-Visible spectrophotometer method: In this work all the fruits and vegetables are fresh which collected from Babylon market. Orange has been contain higher amount of ascorbic acid (18ppm) where Red pepper contain less amount (0.67ppm). As shown in table (1) and figure (2).

Table.1. The total ascorbic acid content in fresh Fruits and Vegetables using UV-Visible spectrophotometer method

| Experiment | sample | Amount Ascorbic acid (mg/L) | SD% |
|------------|------------------|-----------------------------|------------|
| 1 | Red pepper | 0.67 | ± 0.02 |
| 2 | Cabbage | 1.11 | ± 0.04 |
| 3 | Tomato | 1.67 | ± 0.03 |
| 4 | Cantaloupe melon | 2.27 | ± 0.06 |
| 5 | Watermelon | 6.38 | ± 0.02 |
| 6 | Pomegranate | 8.71 | ± 0.09 |
| 7 | Banana | 11.98 | ± 0.12 |
| 8 | Celery | 17.65 | ± 2.31 |
| 9 | Kiwi | 17.93 | ± 2.10 |
| 10 | Orange | 18.00 | ± 3.12 |

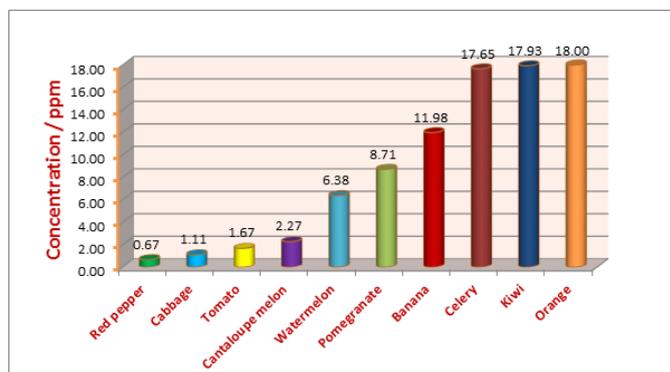


Fig.2. The total ascorbic acid content in fresh Fruits and Vegetables using UV-Visible spectrophotometer method

Determination of vitamin C using titration method: In the second method to determine the value of ascorbic acid was titration method. Orange has been contain higher amount of ascorbic acid (35.72 ppm) where Red pepper contain less amount (14.84ppm).As shown in table(2) and figure(3).

Table.2.The total ascorbic acid content in fresh Fruits and Vegetables using titration method

| Experiment | Sample | Amount Ascorbic acid (mg/L) | SD% |
|------------|------------------|-----------------------------|--------|
| 1 | Red pepper | 14.84 | ± 0.70 |
| 2 | Cabbage | 17.42 | ± 0.73 |
| 3 | Tomato | 22.59 | ± 0.80 |
| 4 | Cantaloupe melon | 25.33 | ± 0.77 |
| 5 | Watermelon | 27.46 | ± 6.01 |
| 6 | Pomegranate | 27.82 | ± 6.33 |
| 7 | Banana | 29.21 | ± 4.23 |
| 8 | Celery | 30.41 | ± 3.51 |
| 9 | Kiwi | 33.71 | ± 3.91 |
| 10 | Orange | 35.72 | ± 3.42 |

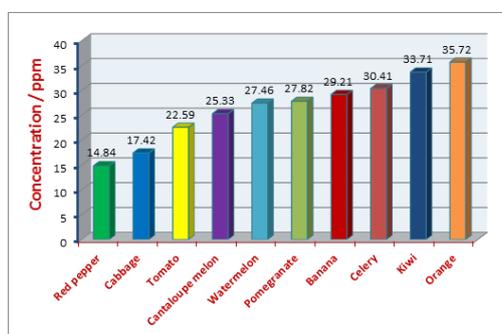


Fig.3.The total ascorbic acid content in fresh Fruits and Vegetables using titration method

The results of the determination of the ascorbic acid content of selected fruits using acetic acid and sulphuric acid as extracting solvents by spectrophotometric and titrimetric methods are shown in tables 1 and 2 respectively. The ascorbic acid content by titrimetric method ranged from 14.84 ± 0.70 mg/L to 35.72 ± 3.42 mg/L, while spectrophotometric method ranged from 0.67 ± 0.02 mg/L to 18.00 ± 3.12 mg/L. This shows that the titrimetric method could be inconsistent and unreliable, and thus cannot be preferred. Higher contents of ascorbic acid (Vitamin C) were obtained with the spectrophotometric method in all the selected fruits and vegetable when compared with the titrimetric method. This shows clearly that the measurement titration method is less sensitive to determine the content of ascorbic acid fruits.

4. CONCLUSION

Spectrophotometric method for determination of ascorbic acid in fruits and vegetables from Hill region with 2,4-DNPH is a simple and reliable method. Comparison of results obtained by Titration method is a good agreement with results obtained by titration method. Orange contain large value of ascorbic acid and less value presence in red pepper using UV-Visible Spectrophotometer and titration method.

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