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A NEW PHOTOMETRIC METHOD OF ASSAY OF VITAMIN C IN TOMATO

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ABSTRACT

Ascorbic acid is an important water soluble vitamin which is present in several natural foods as well as pharmaceutical preparations. In our present study an indirect colorimetric method of estimation of ascorbic acid present in tomato fruits was carried out. The proposed method was to oxidize ascorbic acid present in the sample (tomato) with potassium chromate as oxidizing agent in the presence of a mineral acid and by determining the unreacted amount of potassium chromate photo-metrically by converting it into a violet coloured chromium(VI) complex by reacting with 1,5-diphenylcarbazide. This indirect photo-metric determination of ascorbic acid is more selective and sensitive wherein trace quantities of vitamin C in samples may be determined. The level of acid concentration was also determined which is very accurate. The precision and accuracy of the new method was evaluated by comparing the results with standard method of titration of ascorbic acid against 2,6-dichlorophenol-indophenol (DCPIP).The fruit samples may contain other materials like carbohydrates (sugars), and organic acids along with a trace quantity of vitamin C. The level of interference of such sugars and carboxylic acids in the proposed photometric method of determination of ascorbic acid was also ruled by comparing with standard method.

INTRODUCTION

Food is the very basis of life and is needed for all activities of all living beings. [1, 2] Food chiefly consists of complex organic substances such as carbohydrates, fats and proteins. These basic materials get oxidized to meet most of the energy requirements.

The nutrients are divided into two groups: one is energy yielding dietary components such as carbohydrates, fats and proteins and the other is accessory food factors that include vitamins and inorganic substances like minerals, including trace element. Calcium, chlorine, iron, zinc, copper, sulphur and iodine are important minerals required by living beings. Traces of cobalt, manganese, molybdenum, chromium, fluorine and selenium are also required for the well being of animals.

Vitamins are organic compounds required in small quantities in the diet to maintain various metabolic reactions in tissues. Deficiency of vitamins [3] in foods leads to several deficiency syndromes and diseases in humans and animals.

Vitamins are classified as fat soluble (A, D, E, and K) and water soluble (C and B group) vitamins. Most of the water soluble vitamins form coenzymes that participate in a variety of biochemical reactions while only one fat soluble vitamin (K) has been identified to function as coenzyme. These water soluble vitamins are excreted in urine and they are not toxic to the body and they are not stored in the body in large quantities except vitamin B_{12}.

Most of the biochemical functions of vitamin C [4] are related to its property to undergo reversible oxidation and reduction. Vitamin C is required for bone formation. Ascorbic acid[5] enhances iron absorption by keeping it in the ferrous form. This is due to the reducing property of vitamin C. It enhances the synthesis of immunoglobulins and reduces the risk of cataract formation.

An intake of 60-70mg vitamin C per day will meet the adult requirement. The deficiency of ascorbic acid results in scurvy. This disease is characterized by spongy and sore gums, loose teeth, anemia and swollen joints. Ascorbic acid as such is not toxic, but dehydroascorbic acid (oxidized form of ascorbic acid) is toxic. Further oxalate is a major metabolite of vitamin C.

Oxalate has been implicated in the formation of kidney stones. Hence mega doses of ascorbic acid may lead to oxalate poisoning and kidney stone formation [5].

MATERIALS AND METHODS

Procurement of chemicals

Potassium chromate, diphenylcarbazide, L-Ascorbic acid, Nitric acid were of analytical grade and procured from Southern India Scientific Company, Tiruchirappalli.

Instruments and chemical preparation

Elico photoelectric colorimeter, CL157 was employed for the determination. The green filter having transmission maximum at ~540nm is found suitable for the measurement Analar grade chemicals and redistilled water were used for preparing the reagents.

Titration with 2,6-dichloropheno-indophenol:

Ascorbic acid is oxidized by a coloured dye 2,6-dichloropheno-indophenol to dehydro ascorbic acid. During this process the dye is reduced to a colourless luco compound. So when a sample ascorbic acid is titrated against an aqueous solution of the dye solution which is containing same amount of sodium bicarbonate, appearance of pale pink colour is the end point. The titration of ascorbic acid sample with this dye solution containing ~50mg of dye in 250ml water is very specific with respect to ascorbic acid. To increase the specificity of the reaction and to minimize the effect of interference of other substances the reaction is carried out in an acidic medium. This method is very useful in determining trace quantities of ascorbic acid present in a sample solution.

Estimation of l-ascorbic acid in the tomato sample

Extraction of tomato juice

A ripened tomato fruit of weight 77.172g was taken to prepare the juice. The fruit was cut into four. All the four portions of the fruit were squeezed thoroughly in a mortar with a pestle after adding 50ml of 0.8M nitric acid. The juice was filtered into a 250ml standard volumetric flask. The residue in the mortar was repeatedly squeezed after adding some nitric acid solution and the

liquid portion was filtered into the standard flask. Finally the juice in the volumetric flask was made upto the mark with 0.8M nitric acid. In neutral or in alkaline aqueous solution L-ascorbic acid spontaneously gets oxidized by air to L-dehydroascorbic acid.

Estimation of Ascorbic acid

Tomato juice extract in the range of 1, 2, 5, 10, 15 ml etc were pipetted out into a 25ml standard flask followed by 2.1ml of standard potassium chromate, and 3ml of diphenylcarbazide solution. The mixture was diluted with 0.8M nitric acid up to the mark. The solution was thoroughly shaken for complete development of the colour. The optical density of the coloured solution was measured using a green filter after adjusting the scale to zero. From the optical density, the amount of unreacted potassium chromate was read from the calibration graph. Hence the amount of potassium chromate that reacted with ascorbic acid which was present in 5ml of extract, also the amount of ascorbic acid present in 77.172g of the fruit was calculated. From the values the amount of ascorbic acid that would be present in 100g of the fruit was also calculated.

RESULTS AND DISCUSSION

Table – 1 - Optical Density Of Standard K_2CrO_4

S. No.	Volume of standard K_2CrO_4(ml)	Optical density
1.	0.50	0.25
2.	1.00	0.50
3.	1.50	0.75
4.	2.00	1.00
5.	2.50	1.25

Table – 2 Amounts of Ascorbic Acid Present In Different Aliquots Of Tomato Extract

S. No.	Volume of tomato extract (ml)	Weight of L-Ascorbic acid found (mg)
1.	1.0	0.0117
2.	2.0	0.0234
3.	5.0	0.0582
4.	10.0	0.1170
5.	15.0	0.1756

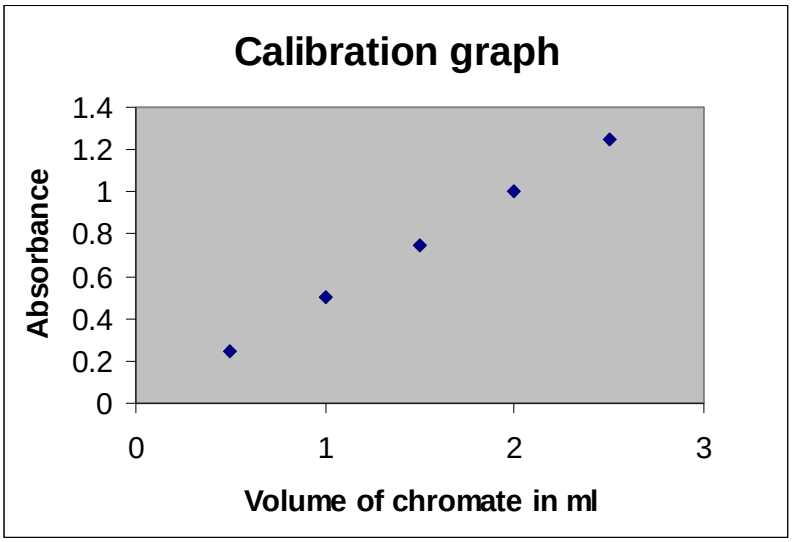
TABLE – 3 – Comparison Of Ascorbic Acid Present In The Tomato To Standard Solution

S. No.	Wt. of ascorbic acid found by the proposed method (mg)	Wt. of ascorbic acid found by the standard method (mg)	Relative error in percentage
1.	0.0117	0.0116	+0.77
2.	0.0234	0.0235	–0.44
3.	0.0582	0.0584	–0.34
4.	0.1170	0.1159	+0.94
5.	0.1756	0.1748	+0.45

The values of relative errors shown in Table 3 point to the fact that the new method is as precise and accurate as the 2, 6-dichlorophenol-indophenol titration method.

A calibration curve was obtained by plotting optical density against the volume of the standard potassium chromate solution in milliliter. The plot is a straight line passing thorough the origin.

FIGURE I



Under the experimental conditions the above calibration graph is linear described by the equation, A = 0.5x. Here A is absorbance and x is the volume of chromate solution containing 4.209 mg of chromium (VI)/ ml.

Sample calculation

Wt. of tomato fruit	=77.172g
Volume of extract prepared taken	=250ml
Volume of tomato extract	=5ml
Absorbance measured	=0.72
Corresponding volume of unreacted K_2CrO_4 read from the calibration curve	=1.425ml
Volume of std. K_2CrO_4 which reacted with ascorbic acid present in 5ml of tomato extract	=2.1–1.425 =0.575ml
/Wt. of K_2CrO_4 reacted with Ascorbic acid present in 5ml of extract	=0.0744x0.575 0.04278mg
Wt. of ascorbic acid present in 5ml of tomato extract	$=\frac{0.04278 \times 528.192}{388.400}$ 0.0582mg
Wt. of ascorbic acid present in 250ml of extract or 77.172g of tomato	$=\frac{0.0582 \times 250}{5}$ =2.91mg
Wt. of ascorbic acid that would be present in 100g tomato	$=\frac{2.91 \times 100}{77.172}$ =3.7708mg.

In the present study the results of investigation on a new colorimetric assay of ascorbic acid in tomato fruit extract were embodied. Vitamin C also known as ascorbic acid is a versatile water soluble vitamin, which plays a significant role in human health. The basis of colorimetric analysis is the variation of intensity of colour of a substance in accordance with concentration.[6,7] Ascorbic acid is a strong reducing agent. The content of ascorbic acid in food materials as well as pharmaceutical preparations (like capsules, tablets, tonics etc) can be estimated based on its reducing properties.[8,9]

A survey of literature shows that the L Ascorbic acid contents of various fruit and vegetables can be determined by several oxidimetric methods excepting the proposed methods. Hence this new, indirect colorimetric method involving chromium(VI)-diphenyl carbozide reagent system was developed. 1,5-diphenyl carbazide has been utilized for the selective and direct colorimetric determination of chromates and dichromates [10]. Diphenyl carbazide may also be used for the determination of chromium in the lower oxidation states by converting it into Cr(VI).

Diluted standard solution of K_2CrO_4 in the range of 0.5, 1.0, 1.5, 2.0, 2.5ml etc. were taken separately in 25ml volumetric flasks. Two milliliter of diphenylcarbazide (DPC) solution in alcohol was added to each standard flask. It was made up to the mark with 0.8M HNO3. Each volumetric flask was stoppered and shaken thouroughly for the uniform development of colour. The cuvette was rinsed with the solution and filled up to the mark and optical density was measured for each case. L-Ascorbic acid present in a known volume of the tomato extr4act is allowed to react with a known volume potassium chromate in acid medium and subsequently the unreacted amount of potassium chromate was made to react with diphenylcarbazide in nitric acid medium. The measurement of optical density of the violet compound produced by the reaction of unreacted potassium chromate with diphenylcarbazide is an important step in the indirect determination of L-ascorbic acid content of the tomato extract. The reaction between the unreacted chromate and diphenylcarbazide (DPC) is as follows:

$$CrO_4^{2-} + DPC \rightarrow Cr^{VI} - DPC \text{ (Violet complex)}$$

Colorimetric method is the newly proposed method of analysis of ascorbic acid in the fruit sample (tomato). Ascorbic acid is a strong reducing agent. Hence various redox methods involving KIO_3, iodine, ceric ammonium sulphate, chloramines-T etc. are frequently used for the

assay of ascorbic acid in samples. However, when vitamin C is in trace quantities in a sample, titration with 2, 6-dichlorophenol-indophenol is largely employed. Vitamin C is a marker chemical. The level of ascorbic acid in a food sample indicates the quality of the food product.

The new method involves the use chromate-diphenylcarbazide reagent system. A known excess of standard potassium chromate solution is used to oxidize L-ascorbic acid to L-dehydro-ascrobic acid present in a given aliquot of the fruit extract. The unreacted amount of potassium chromate is estimated colorimetrically by converting it into a stable soluble violet coloured chromium (VI)-diphenylcarbazide complex and thereby the ascorbic acid content is determined.

Both the oxidation of L-ascorbic acid and the complexation of chromium (VI) with DPC go to completion only in an acid medium. Hence it is important to study and fix the appropriate acid concentration at which the oxidation and complexation reactions are complete and also the colour developed is stable over a period of time. The study shows that 0.8M nitric acid is much suitable for the proposed analysis.

CONCLUSION

Fruits like tomatoes contain good quantities of sugars and carboxylic acids. A study of interference caused by these substances has been made and it is inferred that these substances do not cause interference. The results collected from the proposed colorimetric method are compared with those obtained from the standard volumetric method involving DCPIP reagent. It is found that the precision and accuracy of the new method are as good as those of DCPIP method and hence the proposed method could be readily implemented to assay ascorbic acid in real samples.

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