Colorimeteric Estimation of vitamin-C present in fruits

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Introduction

Vitamin-C or L-Ascorbic acid is very closely related to monosaccharides. It is a water soluble vitamin widely distributed in the plant and animal kingdom. It is of major importance in nutrition, maintenance of good health and in food industry. Ascorbic acid is added to food as a nutrient or as a processing aid in brewing, wine making, bread making, meat curing and freezing of fruits. Vitamin-C is needed for the growth and repair of tissues in all parts of the body. Vitamin-C is essential for the healing of wounds, and for the repair and maintenance of cartilage, bones and teeth. Vitamin-C is one of many natural antioxidants, vitamin-E and β-carotene being two other well known natural antioxidants.

Vitamin-C deficiency can lead to dry and splitting hair; gingivitis (inflammation of the gums) and bleeding gums, rough, dry scaly skin; decreased wound healing rate, easy bruising; nosebleeds; weakened enamel of the teeth; swollen and painful joints; anemia; decreased ability to ward off infection; and possibly weight gain because of slowed metabolic rate energy expenditure. A severe form of Vitamin-C deficiency is known as scurvy. The human body does not synthesize Vitamin-C on its own, nor does it store it. It is therefore important to include plenty of Vitamin-C containing foods in one's daily diet.

Vitamin-C is widely distributed in both plant and animal kingdom, probably in equilibrium with dehydro-L-ascorbic acid. It occurs in significant quantities in vegetables, fruits and animal organs such as liver and kidney but in only small quantities in meat. Vitamin-C is present in mother's milk and, in lower amounts, in raw cows, with pasteurized milk containing only trace amounts. All excess Vitamin-C is disposed of through the urinary system.

Table-1.Vitamin-C in vegetables and fruits.

Vegetables	**mg/100 g of edible portion**	**Fruits**	**mg/100 g of edible portion**
Cabbage	30 – 70	Kiwifruit	80 – 90
Carrot	5 – 10	Lemon	40 – 50
Cucumber	6 – 8	Melons	10 – 15
Cauliflower	50 – 70	Orange	30 – 50
Kale	70 – 100	**Vegetables**	**mg/100 g of edible portion**
Lettuce	10 – 30	Potato	4 - 30
Onion	10 – 15	Pumpkin	15
Pea	8 - 12	Asparagus	15 – 30

Experimental procedure:

Reagents required:

- **Stock ascorbic acid solution:** It is prepared by dissolving 0.1 grams of ascorbic acid in 100 mL of glass distilled water 1 mg of ascorbic acid is present per mL of solution.
- **Standard ascorbic acid solution:** 10 mL of stock solution is diluted to 100 mL with distilled water such that each mL of solution contains 0.1 mg of ascorbic acid. Ascorbic acid solution should be freshly prepared before the analysis.
- **Potassium chromate solution:** Potassium chromate solution is prepared by dissolving 0.0934 grams of pure potassium chromate in 250 mL of distilled water such that the solution contains 0.1 g/L of chromium (VI).
- **Diphenylcarbazide reagent solution (DPC reagent):** The reagent solution is prepared by dissolving 0.0989 grams of sym-diphenylcarbazide in 100 mL of alcohol. The reagent should be prepared freshly before use.
- **0.8M HNO_3 solution:** It is prepared by diluting 10.75 mL of concentrated nitric acid to 250 mL with distilled water.

Sample preparation:

Samples selected for the estimation of ascorbic acid are fruits like lemon, orange, grape, tomato.

Lemon, orange, grape and tomato are crushed and filtered to remove solid waste and the clear solution is diluted with water in 100 mL standard flasks. The dilute solution of each fruit juice is taken for the estimation. Juice of each fruit is taken in triplicate and analyzed.

Plot of standard graph of ascorbic acid:

Different volumes of standard ascorbic acid solution are taken in different 25 mL standard flasks as shown in table-2. To each flask, 0.2 mL potassium chromate solution, 4.5 mL of 0.8M HNO_3 solution, 4.0 mL of diphenylcarbazide reagent solution are added. Then all flasks are made up to mark with distilled water and shaken well for uniform concentration (Table-2). After 15 minutes allowed for full colour development, the intensity of pink colour developed is measured at λ_{max} using blank. The λ_{max} is determined by measuring the intensity of colour at different wavelengths using blank (Table-2 and 5).

Then ascorbic acid solution of known concentration is taken and the colour is developed by adding the all the reagents as described above and the intensity of colour is measured. Then the concentration of ascorbic acid is determined using standard graph to verify the validity of the method (Table-3).

Estimation of ascorbic acid in fruits:

Later different aliquots of fruit juices of different fruits mentioned above are taken in different 25 mL standard flasks as shown in table-2. After adding all the reagents, the mixture is made up to mark with distilled water and shaken well for uniform concentration. Then the intensity of colour is measured at λ_{max}. The concentration of Vitamin-C in different fruits is calculated from the calibration curve (Tables 4).

Table-2: Calibration curve:

Trial No.	Vol. of Vitamin-C solution	Conc. Of Vitamin-C (mg)	Vol. of K_2CrO_4 solution	Vol. of 0.8M HNO_3 solution	Vol. of DPC reagent	Vol. of Water	Absorbance
1	0.5	0.05	0.2	4.5	4.0	15.8	0.4
2	1.0	0.1	0.2	4.5	4.0	15.3	0.37
3	1.5	0.15	0.2	4.5	4.0	14.8	0.32
4	2.0	0.2	0.2	4.5	4.0	14.3	0.28
5	2.5	0.25	0.2	4.5	4.0	13.8	0.25
6	3.0	0.3	0.2	4.5	4.0	13.3	0.21
7	3.5	0.35	0.2	4.5	4.0	12.8	0.18
8	4.0	0.4	0.2	4.5	4.0	12.3	0.14
9	4.5	0.45	0.2	4.5	4.0	11.8	0.1
10	5.0	0.5	0.2	4.5	4.0	11.3	0.07

Table-3: Verification of standard graph:

Trial No.	Vol. of grape solution	Vol. of K_2CrO_4 solution	Vol. of 0.8M HNO_3 solution	Vol. of DPC reagent	Vol. of Water	Absorbance	*Conc. From graph*	*Conc. expected*	*% recovery*
1	0.5	0.2	4.5	4.0	15.8	0.39	0.045	0.05	90
2	1.5	0.2	4.5	4.0	14.8	0.32	0.14	0.15	93.3
3	3.0	0.2	4.5	4.0	13.3	0.2	0.29	0.3	96.7
4	4.5	0.2	4.5	4.0	11.8	0.12	0.43	0.45	95.6

Table-4: Estimation of ascorbic acid in fruits:

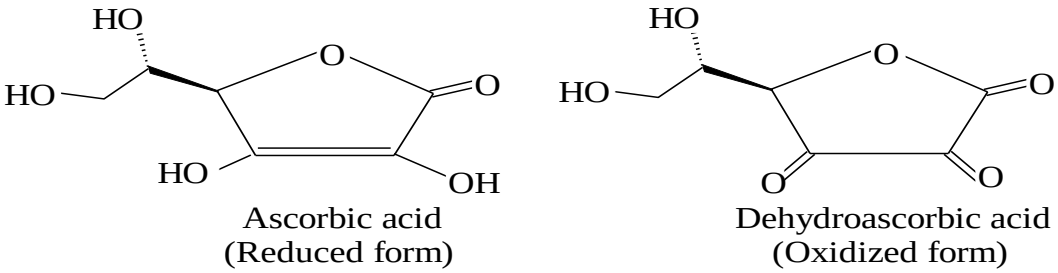
Name of fruit	Trial No.	Vol. of grape solution (ml)	Vol. of K_2CrO_4 solution	Vol. of 0.8M HNO_3 solution	Vol. of DPC reagent	Vol. of Water	Absorbance	*Conc. From graph*	*Total amount of vit.C (mg/100g)*	*Average*
Grape	1	1.0	0.2	4.5	4.0	15.3	0.4	0.03	60.0	62.2 mg
	2	2.0	0.2	4.5	4.0	14.3	0.38	0.07	60.0	
	3	3.0	0.2	4.5	4.0	13.3	0.35	0.1	66.7	
Orange	1	1.0	0.2	4.5	4.0	15.3	0.41	0.02	40.0	42.23 mg
	2	1.5	0.2	4.5	4.0	14.8	0.395	0.035	46.7	
	3	2.0	0.2	4.5	4.0	14.3	0.39	0.04	40.0	
Lemon	1	2.0	0.2	4.5	4.0	14.3	0.39	0.04	40.0	43.1 mg
	2	4.0	0.2	4.5	4.0	12.3	0.36	0.085	42.5	
	3	6.0	0.2	4.5	4.0	10.3	0.325	0.14	46.7	
Tomato	1	2.0	0.2	4.5	4.0	14.3	0.4	0.025	12.5	11.94 mg
	2	4.0	0.2	4.5	4.0	12.3	0.39	0.04	10.0	
	3	6.0	0.2	4.5	4.0	10.3	0.365	0.08	13.3	

Results and Discussion:

The principle of colorimetric method for the estimation of ascorbic acid is based on the reaction of ascorbic acid with potassium chromate. Potassium chromate oxidizes ascorbic acid dehydroascorbic acid. Then the excess of potassium chromate is treated with diphenylcarbazide $[CO(NHNHC_6H_5)_2]$ which forms pink coloured complex.

$$2\ CrO_4^{2-} + 3\ C_6H_8O_6 + 10\ H^+ \longrightarrow 2\ Cr^{3+} + 3\ C_6H_6O_6 + 8\ H_2O$$

$$\text{Unreacted } CrO_4^{2-} + \text{sym-DPC} \xrightarrow{H^+} \text{Cr-DPC complex}$$



Ascorbic acid (Reduced form) Dehydroascorbic acid (Oxidized form)

Oxidized and reduced forms of ascorbic acid.

Fig-1

The intensity of colour is measured at λ_{max}. The λ_{max} is determined by using solution of lower concentration of ascorbic acid against blank solution (Table-5). Absorption maximum occurs at 520 nm (Fig-2). Then intensity of all solutions is measured at 520 nm. Standard graph is plotted by taking the concentration of ascorbic acid against absorbance. As the concentration of ascorbic acid increases, absorbance decreases (Fig-3).

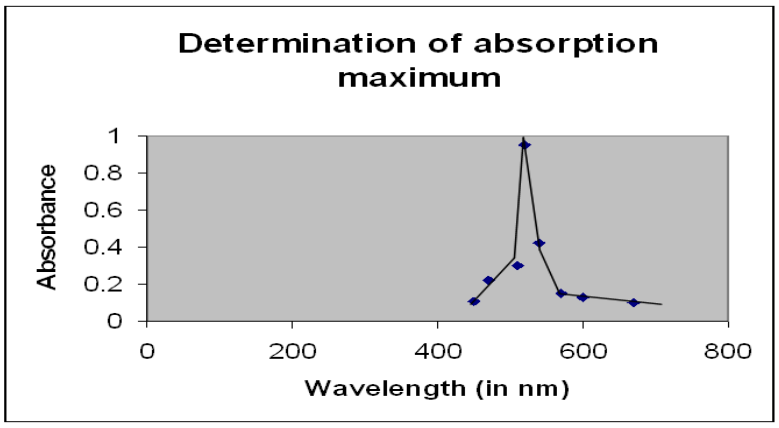


Fig-2

The standard graph is verified by taking known concentration of ascorbic acid solution and absorbance is measured. The concentration of ascorbic acid is calculated using standard graph. The results show 95.8% recovery (Table-3). This shows that this method can be conveniently used for the estimation of ascorbic acid from various sources.

Table-5: Determination of λ_{max}

Wavelength	Absorbance
450	0.11
470	0.22
510	0.30
520	0.95
540	0.42
570	0.15
600	0.10
670	0.10

λ_{max}: 520 nm.

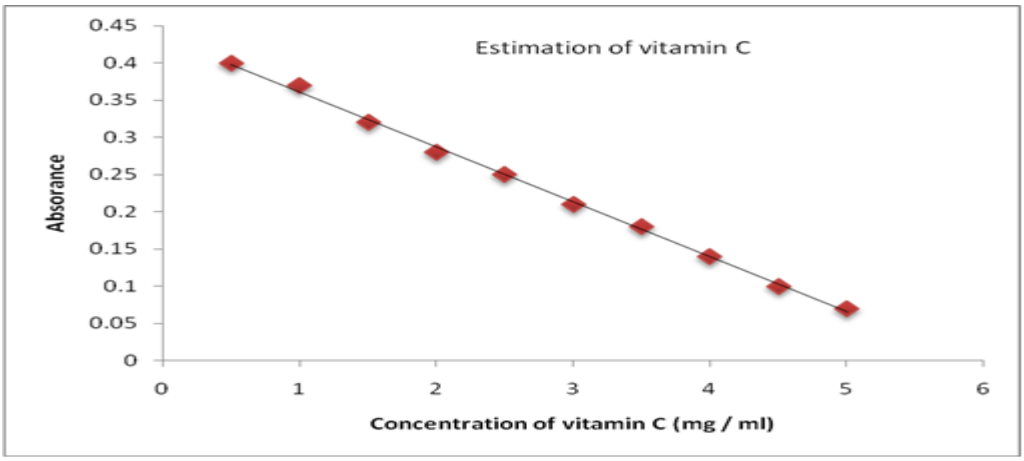


Fig-3

Conclusions:

- The principle of colorimetric method for the estimation of ascorbic acid is based on the reaction of ascorbic acid with potassium chromate. Potassium chromate oxidizes ascorbic acid dehydroascorbic acid. Then the excess of potassium chromate is treated with diphenylcarbazide [$CO(NHNHC_6H_5)_2$] which forms pink coloured complex. The absorbance maximum for this complex is 520 nm and the intensity of colour of all other solutions is measured at 520 nm.
- Standard graph is plotted by taking the concentration of ascorbic acid against absorbance. As the concentration of ascorbic acid increases, absorbance decreases. The standard graph is verified by taking known concentration of ascorbic acid solution and absorbance is measured. The concentration of ascorbic acid is calculated using standard graph. The results show 95.8% recovery.
- Colorimetric method is used for the estimation of ascorbic acid content in different fruits and tablets.
- Ascorbic acid in grapes is found to be 62.2 mg /100 g.
- Ascorbic acid in oranges is found to be 42.23 mg /100 g.
- Ascorbic acid in lemon is found to be 43.1mg /100 g.
- Ascorbic acid in tomato is found to be 11.94 mg /100 g.

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