EXPERIMENT 1 DETERMINATION OF ASCORBIC ACID BY TITRIMETRIC AND COLORIMETRIC METHODS

Structure

1.1 INTRODUCTION

Fruits and vegetables are important sources of ascorbic acid (vitamin C). Ascorbic acid being unstable under different storage and processing conditions, it is important to know its residual content in food products. The most satisfactory chemical method of estimation is based on the reduction of 2,6-dichlorophenol indophenol by ascorbic acid. This can be performed either by titration or by colorimetric method. In this experiment you will be learning both the methods.

Objectives

After studying and performing this experiment, you should be able to:

- prepare different types of food samples for ascorbic acid estimation;
- determine the ascorbic acid content by dye titration method; and
- determine the ascorbic acid content by xylene extraction and colorimetric method.

1.2 EXPERIMENT 1a: DYE TITRATION METHOD

1.2.1 Principle

2,6-dichlorophenol indophenol dye, which is blue in alkaline solution and red in acid solution, is reduced by ascorbic acid to a colourless form. The reaction is quantitative and can be performed by titration. This reaction is practically specific for ascorbic acid in fresh fruits and vegetables. Sulphur dioxide present in products like squashes can deduce the dye and thus interferes in the estimation. Condensing SO_2 with formaldehyde can eliminate this interference.

1.2.2 Requirements

Apparatus/Glassware

Microburette, 10 ml capacity with 0.05 ml sub-graduations

Burette stand

Volumetric flask, 100 ml

Pipette, 1 ml

Conical flask, 100 ml

Analytical balance, 0.1 mg sensitivity

Whatman No.1 filter paper circles

Glass funnel, 2" dia.

Chemicals and Reagents

i) 3% (w/v) Metaphosphoric acid (HPO_3); Prepare by dissolving the sticks or pellets of HPO3 in distilled water.

ii) Ascorbic acid standard: Weigh accurately 100 mg of L-ascorbic acid and make up to 100 ml with 3% HPO_3 solution. Dilute 5 ml to 50 ml with 3% HPO_3 solution (1 ml = 0.1 mg of ascorbic acid).

iii) Dye solution: Dissolve 50 mg of the sodium salt of 2,6-dichlorophenol indophenol in approximately 150 ml of hot distilled water containing 42 mg of sodium bicarbonate. Cool, filter and dilute with distilled water to 200 ml. Store in a refrigerator and standardize every day.

iv) Formaldehyde, 40% solution.

v) Conc. Hydrochloric acid.

1.2.3 Procedure

Standardization of Dye

Pipette out 5 ml of the standard ascorbic acid solution into a 100 ml conical flask and add 5 ml of the 3% HPO_3 solution. Fill the microburette with the dye solution. Titrate the ascorbic acid solution with the dye solution to a pink colour, which should persist for 15 sec. Note the Titre value. Calculate the dye factor.

Volume of ascorbic acid solution taken for titration = 5 ml

Volume of dye solution required (titre) = V = —— ml

Dye factor = mg of ascorbic acid per ml of the dye

Since 5 ml of the standard ascorbic acid solution contains 0.5 mg ascorbic acid:

$$\text{Dye factor} = \frac{0.5}{\text{Titre}} = \frac{0.5}{V} = \text{mg ascorbic acid per ml dye}$$

Preparation of Sample

Juices and liquid products: Take 10-20 g sample and make up to 100 ml in a volumetric flask with 3% HPO_3 solution. Filter through a Whatman No. 1 filter paper.

Solid or semi-solid products: Blend 10-20 g sample with 3% HPO_3 solution and make up to 100 ml in a volumetric flask with 3% HPO_3 solution. Filter through a Whatman No. 1 filter paper.

Titration

Pipette out 2-10 ml of the sample extract into a 100 ml conical flask and titrate against the dye solution as above. The volume of the sample should be such that the tire value is in the range of 3-5 ml.

If the sample contains sulphur dioxide, to the pipetted out sample extract add 1 ml of the formaldehyde solution and 0.1 ml HCl, keep for 10 min and perform the titration.

1.2.4 Observations

Weight of sample taken for extraction with HPO_3 = W = —— g

Volume of the sample made up with HPO_3 solution = 100 = —— ml

Volume of sample extract taken for dye titration = V_1 = —— ml

Volume of dye required (titre) = V_2 = —— ml

1.2.5 Calculations

Ascorbic acid in V_1 ml of the sample extract = dye factor × V_2 = mg

Therefore, ascorbic acid in 100 ml of the extract = $\frac{\text{Dye factor} \times V_2 \times 100}{V_1}$ = mg

Since W g sample was made up to 100 ml, ascorbic acid content of the sample (mg per 100 g)

$$= \frac{\text{Dye factor} \times V_2 \times 100 \times 100}{V_1 \times W} = \frac{\text{Dye factor} \times V_2 \times 10{,}000}{V_1 \times W}$$

1.2.6 Result

Ascorbic acid content of the sample = ... mg per 100 g.

1.3 EXPERIMENT 1b: XYLENE EXTRACTION METHOD

1.3.1 Principle

This method is based on measurement of the extent to which a 2,6-dichlorophenol indophenol solution is decolourised by ascorbic acid in sample extracts and in standard ascorbic acid solutions. The excess dye is taken up in xylene and colour measured in a colorimeter at 520 nm. This method is particularly suitable for stored products in which considerable interfering substances are present.

1.3.2 Requirements

Colorimeter with sufficient number of sample tubes

Analytical balance, 0.1 mg sensitivity

Volumetric flask, 100 ml, and 1000 ml

Pipette, 10 ml

Conical flasks, 50 ml glass stoppered

Funnel

Whatman No.1 filter circles

Reagents

i) Acetate buffer- pH 4: Mix 500 ml of 50% sodium acetate ($CH_3COONa.3H_2O$) with 500 ml of glacial acetic acid.

ii) Dye: Dissolve 125 mg of 2,6-dichlorophenol indophenol (sodium salt) in warm distilled water, cool , make up to 100 ml in a volumetric flask and filter (stock solution). Dilute 18 ml to 100 ml with water. 1 ml of this solution should be equal to 0.1 mg of ascorbic acid. The stock solution of the dye may be stored in a refrigerator for a week.

iii) Meta phosphoric acid solution (3%): Dissolve 15 g of sticks or pellets of HPO_3 in distilled water and dilute to 500 ml.

iv) Standard ascorbic acid solution: Weigh exactly 100 mg of ascorbic acid and make up to 100 ml with 3% HPO_3 solution. Dilute 10 ml to 100 ml (1 ml = 0.1 mg ascorbic acid).

v) Xylene.

vi) Formaldehyde 40%.

vii) Anhydrous sodium sulphate.

1.3.3 Procedure

Sample extraction procedure followed for the titration method may be followed for this method also.

Standard Curve

Pipette out 0.0, 0.50, 0.75, 1.0, 1.5 and 2.0 ml of the standard ascorbic acid solution into 50 ml stoppered conical flasks. Make up the total volume in each flask to 2 ml with 3% HPO_3 solution. Add 1 ml water, 2 ml acetate buffer, 3 ml dye solution and 15 ml xylene in rapid succession. Stopper the conical flasks and shake vigorously for 10 sec to extract the excess dye into the xylene. Allow the layers to separate. With a pipette completely draw out the water layer below the xylene layer and discard. Add a small quantity (0.5-1 g) of anhydrous Na_2SO_4 to the xylene layer to remove traces of moisture. Transfer the xylene extracts to the colorimeter tubes and measure the absorbance at 520 nm. Set the instrument to 100% transmittance using xylene as blank. Plot the absorbance values (A) against ascorbic acid (mg) on a graph paper to get the standard curve. You will see that as the concentration of ascorbic acid in the reaction mixture increases, the absorbance value decreases.

Vol. of Ascorbic Acid (ml)	Ascorbic Acid (mg)	Absorbance (A)
0.0	0.00	
0.5	0.05	
0.75	0.075	
1.0	0.10	
1.5	0.15	
2.0	0.20	

Sample

Take 2 ml sample extract in a stoppered conical flask, add 2 ml of buffer, 1 ml of 40% formaldehyde and mix. Allow to stand for 10 min. Then add 3 ml dye solution, stopper and shake for 10-15 sec. Follow the remaining steps as done in the case of standard curve preparation. From the standard curve note the ascorbic acid content (mg) in the 2 ml sample extract taken for the estimation.

1.3.4 Observations

Absorbance of the xylene extract = A_1

Corresponding ascorbic acid content from the standard curve = W_1 = —— mg

Weight of the sample taken for extraction = W_2 = —— g

Volume of the sample made up for ascorbic acid extraction = 100 = —— ml

1.3.5 Calculations

From the data, W1 mg of ascorbic acid is present in 2 ml of the sample extract.

As W g of the sample was made up to 100 ml for extraction of ascorbic acid, the ascorbic acid content of the sample (mg per 100 g)

$$= \frac{W_1 \times 100 \times 100}{2 \times W_2} = \frac{W_1 \times 5000}{W_2}$$

1.3.6 Result

Ascorbic acid of the sample = ... mg per 100 g.

1.4 PRECAUTIONS

The general precautions mentioned in the course 'Introduction' and those indicated in the experiments should be followed meticulously.

The colorimeter and the sample tubes should be handled with care.