Indira Gandhi
National Open University
School of Agriculture

# BPVI-037
# Food Quality Testing and Evaluation

## PRACTICAL MANUAL

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# BPVI-037 FOOD QUALITY TESTING AND EVALUATION

The Food Quality Testing and Evaluation Lab course has been designed to make you familiar with the various analytical methods used in food analysis, especially for determining some important individual constituents and additives. One experiment on testing of sensory quality of foods and food products is also included. The basic principles on which the analytical procedures are based are explained under each experiment.

There are eleven experiments in this course. Some of them are clubbed together for convenience. For example, Experiment-1 'Determination of ascorbic acid' has 2 sub-experiments viz. 1a: Ascorbic acid by titration method and 1b: Ascorbic acid by colorimetric method.

While performing the experiments, the following general precautions and care have to be taken for obtaining reliable results. Since these precautions are general to all the analytical methods, it will be redundant to repeat them under each method.

**Sampling:** Foods and food products are highly variable in composition. In fresh foods like a fruit or a vegetable, the composition varies from the outer portion to inner portion and also from the stem end to the flower end. All processed food products are also not uniform. For example, in a pickle, the composition of the fruit/vegetable portion is different from the gravy. Therefore, proper sampling of the product is very important. A sample, which will represent a product, can be obtained by blending a sufficiently large quantity of the product in a blender and drawing out a sample from that. Wherever, blending of a product as such is difficult, known quantity of water or the recommended solvent has to be added. Details of such instances are given under the respective experiments.

**Cleanliness:** The general and measuring glassware like conical flasks, beakers, dishes, funnels, measuring cylinders, pipettes, burettes etc. used for the analytical work should be thoroughly cleaned using a cleaning solution followed by washing with tap water and distilled water and finally dried.

**Accuracy:** Results of some of the experiments in this course are expressed as mg per 100 g, mg per Kg (ppm) or even µg per 100 g. Therefore, the accuracy and precision with which the experiments have to be performed are higher than those experiments under Course III: Chemistry and Physiology. The sample size to be taken has been indicated under each experiment.

### General Precautions

1. Wear a lab coat or apron while performing experiments.
2. Handle strong acids, alkalies, and poisonous chemicals with care.
3. Never pipette strong acids, alkalies, poisonous solutions or most of the solvents using mouth. Use a vacupette or in most cases it will be sufficient to measure using a measuring cylinder.

### Objectives

After reading this course and carrying out the experiments set for you to do, you should be able to:

- Carry out the individual experiments,
- Record the observations, and
- Calculate and interpret the results,

## Study Guide

This laboratory course involves 8 credits of intense work. You would be required to do 11 experiments described in this Laboratory Manual. Each of the experiments would be graded and you would have to appear for the *viva-voce* also. Seventy percent marks are reserved for performing these set experiments. On the last day, you would be assigned one or two experiments, which would be similarly graded. 30% marks are reserved for the assigned experiment(s).

## Laboratory Notebook

An important part of your scientific training is the maintenance of a complete and up to date record of your laboratory work. For writing and reporting experimental data, laboratory chemistry notebooks are available in the market. Use a 100-page chemistry notebook for this laboratory course.

You should prepare the page for recording an experiment before you come to the lab. For each experiment, you should write down the title of the experiment, important chemical reactions involved, procedures, observations and results. The laboratory notebook must be submitted to the counsellor for correction and grading. Marks have been allocated for doing the experiments and for recording them properly.

We hope you will enjoy the course. We want you to experience the thrills of learning by doing. There is no better way to learn. Wish you best of luck.

# EXPERIMENT 1 DETERMINATION OF ASCORBIC ACID BY TITRIMETRIC AND COLORIMETRIC METHODS

### Structure

1.1 Introduction
Objectives

1.2 Experiment 1a: Dye Titration Method
1.2.1 Principle
1.2.2 Requirements
1.2.3 Procedure
1.2.4 Observations
1.2.5 Calculations
1.2.6 Result

1.3 Experiment 1b: Xylene Extraction and Colorimetric Method
1.3.1 Principle
1.3.1 Requirements
1.3.1 Procedure
1.3.1 Result

1.4 Precautions

## 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

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

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.

# EXPERIMENT 2 DETERMINATION OF SODIUM CHLORIDE

**Structure**

2.1 Introduction
Objectives
2.2 Experiment
2.2.1 Principle
2.2.2 Requirements
2.2.3 Procedure
2.2.4 Observations
2.2.5 Calculations
2.2.6 Result
2.3 Precautions

## 2.1 INTRODUCTION

Salt (sodium chloride) is an important ingredient in several food products like pickles, chutneys, and sauces. Brine (dilute salt solution) is the common covering liquid for most of the low acid canned products like vegetables. In products like "pickle in brine", the minimum salt content has been specified.

The approximate salt content in brine solutions can be measured using a salinometer (hygrometer). However, for more accurate determination of sodium chloride, silver nitrate titration method is mostly followed.

**Objectives**

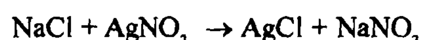
After studying and performing this experiment, you should be able to:

- determine the salt content of food products.

## 2.2 EXPERIMENT

### 2.2.1 Principle

When a sample extract containing sodium chloride to which a few drops of potassium chromate solution is added, is titrated with standard silver nitrate solution, silver nitrate precipitates chloride as silver chloride. Immediately on completion of the precipitation reaction, the excess of silver nitrate reacts with potassium chromate forming reddish brown silver chromate, which is the end point. The quantity of silver nitrate used for the precipitation is the measure of the sodium chloride content of the sample.



$$NaCl + AgNO_3 \rightarrow AgCl + NaNO_3$$

### 2.2.2 Requirements

**Glassware and Other Items**

Chemical balance
Burette, 25 ml – 1
Conical flask, 250 ml – 2
Measuring cylinder
Whatman No.1 filter paper circles,
Funnel, 4 inch – 2

**Reagents**

5% Potassium chromate solution (indicator)

0.1N Silver nitrate solution

Calcium carbonate powder

### 2.2.3 Procedure

Weigh 25 to 50 g of homogenized sample depending on the salt content. Dilute with distilled water and neutralize with 0.1N sodium hydroxide solution using phenolphthalein as indicator. Transfer to a 250 ml volumetric flask, make up to volume, shake and filter. Titrate an aliquot with 0.1 N silver nitrate solution adding about 1ml of 5% aqueous potassium chromate solution as indicator. Note the volume of silver nitrate solution required to produce the reddish brown end point colour. Carry out a blank titration with distilled water of same volume as the sample aliquot.

### 2.2.4 Observations

| | | |
|---|---|---|
| Weight of the sample | = W | = ——— g |
| Volume made up | = V | = ——— ml |
| Volume taken for titration | = $V_1$ | = ——— ml |
| Volume of silver nitrate solution required for sample | = $V_2$ | = ——— ml |
| Volume of silver nitrate required for blank titration | = $V_3$ | = ——— ml |
| Normality of the $AgNO_3$ solution | = N | |

### 2.2.5 Calculations

1000 ml 1 N $AgNO_3$ solution = 1 g mole of sodium chloride = 58.45 g.

Therefore, % NaCl in the sample =

$$\frac{58.45\,(\text{sample titre - blank titre})\,N \times \text{volume made up} \times 100}{1000 \times \text{aliquot volume taken for titration} \times \text{weight of sample}}$$

i.e. % sodium chloride in the sample = $\frac{58.45\,(V_2 - V_3)\,N \times V}{10 \times V_1 \times W}$

### 2.2.6 Result

Salt content in the sample = .................. Percent.

## 2.3 PRECAUTIONS

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

Handle silver nitrate and its solution with care. They can leave permanent stain on the skin and cloth.

# EXPERIMENT 3 DETERMINATION OF TOTAL CAROTENOIDS AND BETA-CAROTENE BY COLORIMETRIC METHOD

**Structure**


3.1 Introduction

Objectives

3.2 Experiment: Total Carotenoids and Beta-carotene Estimation

3.2.1 Principle

3.2.2 Requirements

3.2.3 Procedure

3.2.4 Observations

3.2.5 Calculations

3.2.6 Result

3.3 Precautions


## 3.1 INTRODUCTION

Carotenoids are a group of yellow, orange and orange-red fat-soluble pigments widely distributed in nature. The carotenoids are of great nutritional importance as some of them are converted into vitamin A. Several fruits and vegetables especially the leafy vegetables are good sources for carotenoids. Therefore, estimation of their concentration in fresh and processed foods is important.

Extracting into solvents like petroleum ether and measuring the colour at 452 nm in a colorimeter most commonly estimate total carotenoids in food materials. Beta-carotene is chromatographically separated from total carotenoids on a suitable adsorbent and colorimetrically estimated.

**Objectives**

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

- extract carotenoid pigments from food materials;
- separate beta-carotene from the total carotenoids chromatographically; and
- estimate them colorimetrically.

## 3.2 EXPERIMENT: TOTAL CAROTENOIDS AND BETA-CAROTENE ESTIMATION

### 3.2.1 Principle

Carotenoid pigments (carotenes, xanthophylls and santhophyllesters) being fat-soluble substances, can be extracted into water immiscible solvents like petroleum ether. The absorbance of the extract is measured in a colorimeter or spectrophotometer, and the carotenoids concentration is calculated using a standard curve. Beta-carotene can be separated from total carotenoids extract chromatographically on a magnesium oxide-supercel column and separately estimated.

### 3.2.2 Requirements

Apparatus

Colorimeter or specrophotometer

Chromatographic column, 150 × 19 mm (ID) glass tubes with constriction at one end to attach 3 mm glass tubing. The column should be fixed to a rubber cork, which should fix to a 100 ml Buchner flask.

Plunger for the preparation of the adsorption column.

Buchner flask, 100 ml –2

Suction pump

Analytical balance, 0.1 mg sensitivity

Pestle and mortar

Volumetric flask, 100 ml –6

——— do —— , 250 ml –1

——— do ———, 25 ml –2

Pipettes, 5 and 10 ml

Conical flask, 250 ml –2

Funnels, 3 inch –2

Separating funnel, 250 ml –2

**Reagents**

*Petroleun ether (b.p. 65-70$^0$C)*

Acetone

Chloroform

Anhydrous sodium sulphate

Adsorbent: Mix one part of magnesium oxide (MgO) with three parts of supercel.

3% acetone in petroleum ether

Sea sand, purified

Surgical cotton

### 3.2.3 Procedure

**Standard Curve of β-carotene**

Weigh accurately 25 mg of β-carotene and dissolve in 2.5 ml chloroform and make up to 250 ml with petroleum ether (1 ml = 0.1 mg or 100 μg). Dilute 10 ml of this solution to 100 ml with petroleum ether in a volumetric flask (1 ml = 10 μg). Pipette 5, 10, 15, 20, 25 and 30 ml of this solution to separate 100 ml volumetric flasks, each containing 3 ml acetone and dilute to mark with petroleum ether. The concentration of β -carotene in these solutions will be 0.5, 1.0, 1.5, 2.0, 2.5 and 3.0 μg per ml. Measure the absorbance of the solutions at 452 nm using 3% acetone in petroleum ether as blank. Draw a graph by plotting absorbance against concentration. Record the data as follows.

| β-carotene (μg/ ml) | Absorbance(A) |
|---|---|
| 0.5 | |
| 1.0 | |
| 1.5 | |
| 2.0 | |
| 2.5 | |
| 3.0 | |

**Sample Extraction**

Weigh a well-blended sample (5 to 25 g) containing 10 to 500 μg total carotenoids. Grind in a pestle and mortar with acetone adding small quantity of pure sand. Filter through cotton into a conical flask. Continue extraction and filtration till the residue is colourless. Transfer the combined filtrate to a separating funnel. Add 10 to 15 ml petroleum ether followed by distilled water to transfer the pigments to the petroleum ether phase. Drain out the aqueous phase and filter the petroleum ether extract through anhydrous sodium sulphate. Make up the petroleum volume of the ether extract to 25 ml in a volumetric flask with petroleum ether. Measure the absorption of the solution at 452 nm. Calculate the total carotenoids contents using the standard curve. The results are expressed in terms of β-carotene as μg per 100 g of the material.

**Chromatographic Separation of β-carotene**

Preparation of column: Attach the column to a Buchner flask, apply vacuum and pack the glass column tightly with the adsorbent to a height of about 10 cm. alternatively, press the adsorbent, 2-3 times with a plunger to ensure a tight column. Add anhydrous $Na_2SO_4$ to the top of the column to about 1 cm height.

Sample adsorption and elution: Wash the column with 25 to 50 ml petroleum ether with suction. When the petroleum layer has almost reached the $Na_2SO_4$ surface, disconnect suction pump and attach the column tube to another clean and dry Buchner flask. Pipette out 5 to10 ml of the sample extract into the column and apply suction. Wash the column continuously with 3% acetone in petroleum ether (eluent) taking care not to allow the solvent layer to go below the $Na_2SO_4$ layer. β-carotene moves out of the column prior to all other pigments. When the β-carotene band has flowed out completely, disconnect suction and transfer the contents of the Buchner flask to a volumetric flask and make up to volume with the eluent. Measure the absorbance of the solution at 452 nm using 3% acetone in petroleum ether as blank.

### 3.2.4 Observations

Weight of sample taken for carotenoids extraction = W = —— g

Volume of the petroleum ether extract of the sample = V = —— ml

Absorbance of the solution = A

Concentration of carotenoids in the solution (from std. curve) = C = μg/ml

Volume of the petroleum ether extract taken for

Chromatography = $V_1$ = —— ml

Volume of the β- carotene band made up to = $V_2$ = —— ml

Absorbance of the β-carotene extract = $A_1$

Concentration of β-carotene in the solution (from std. curve) = $C_1$ = — μg/ml

### 3.2.5 Calculations

**Total Carotenoids**

**Concentration total carotenoids in the petroleum ether extract = C µg/ ml**

Therefore, total carotenoids content in V ml of the petroleum ether extract

$= C \times V$ µg

$C \times V$ µg carotenoids are present in W g of the sample

Therefore, total carotenoids content in the sample $= \frac{C \times V \times 100}{W}$ µg per 100 g

**β-carotene**

**Concentration of β-carotene in the β-carotene eluate = $C_1$ µg/ml**

Therefore, β-carotene content in $V_2$ ml of the eluate $= C_1 \times V_2$ µg

$C_1 \times V_2$ µg of β-carotene is present in V1 ml of the extract taken for chromatography.

Therefore, β-carotene content in the sample $= \frac{C_1 \times V_2 \times V \times 100}{V_1 \times W}$ µg per 100 g

### 3.2.6 Result

Total carotenoids content of the sample =........ µg per 100 g.

β-carotene content of the sample = µg per 100 g.

## 3.3 PRECAUTIONS

β-carotene is unstable to light and susceptible to air-oxidation. Therefore, the sample extracts should be prevented from oxidation and light.

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

Never handle petroleum ether near a flame. The solvents should be handled only in a well-ventilated room or inside a hood with exhaust. Avoid inhaling the solvents directly.

# EXPERIMENT 4 DETERMINATION OF SULPHUR DIOXIDE

### Structure

4.1 Introduction
Objectives

4.2 Experiment
4.2.1 Principle
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4.3 Precautions

## 4.1 INTRODUCTION

Sulphur dioxide and sulphites are versatile food preservatives having several beneficial functions. Sulphur oxide added to food products as preservative may exist as undissociated sulphurous acid, as free bisulphite ion, as free sulphite ion, and/or as combined $SO_2$ in the form of hydroxy sulphonates. However, they can cause harmful effects if consumed in higher quantities. Therefore, like for all other preservatives, maximum permissible limits of sulphites in foods have been laid down. Besides, sulphites are not permitted in all foods.

There are two methods used for the estimation of sulphites in foods. Both of them make use of the reducing property of sulphur dioxide. In one method, iodine is used to oxidize sulphur dioxide (sulphurous acid to sulphuric acid in aqueous solution) and in the other method, hydrogen peroxide is used for the oxidation reaction after liberating sulphur dioxide from the product. The latter method is more reliable and hence followed widely.

### Objectives

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

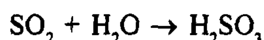
- estimate the sulphur dioxide content of food products by the distillation method.

## 4.2 EXPERIMENT

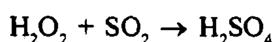
### 4.2.1 Principle

This method measures total sulphur dioxide in food products. Sulphites present in the product are liberated as sulphur dioxide by boiling with hydrochloric acid. The liberated sulphur dioxide is absorbed in hydrogen peroxide solution, which oxidizes it to sulphuric acid. Sulfite content is directly related to generated suphuric acid, which is determined by titration with standard sodium hydroxide solution.

The reactions involved are:



$SO_2 + H_2O \rightarrow H_2SO_3$



$H_2O_2 + SO_2 \rightarrow H_2SO_4$

### 4.2.2 Requirements

**Apparatus**

a) All glass distillation apparatus for determination of sulphur dioxide shown in the diagram below.

**Diagram: SR p 307**

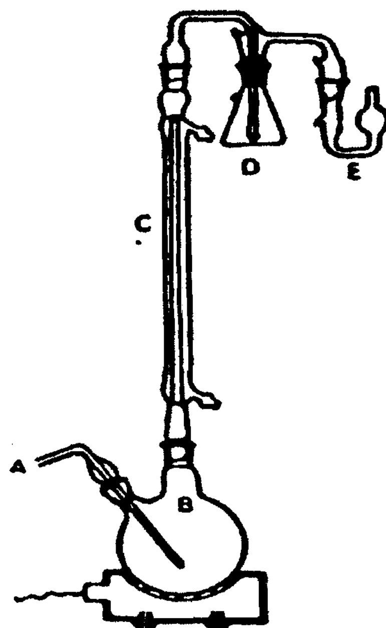



**Figure 4.1: All Glass Distillation Apparatus for Determination of Sulphur Dioxide. A) Glass Inlet Tube, B) 500ml Round-Bottomed Flask, C) Condenser, D) 250 ml Conical Flask, E) Trap**

b) Burette: - 10 ml

**Reagents**

a) **Aqueous Hydrochloric Acid:** -4M. For each analysis, prepare 90 ml solution by adding 30 ml HCl to 60 ml deionized water.

b) **Methyl Red Indicator:** -Dissolve 250 mg methyl red in 100 ml ethanol.

c) **0.05 N NaOH Solution.**

d) **3% Hydrogen Peroxide Solution:** -. For each analysis, dilute 3 ml reagent grade 30% $H_2O_2$ to 30 ml with distilled water. Just prior to use, add 3 drops methyl red indicator and titrate with 0.01N NaOH to yellow end point.

e) **Nitrogen Gas:** -High purity, used with regulator to maintain flow of 200 ml/min.

### 4.2.3 Procedure

Circulate cold water through condenser of the distillation apparatus. Add from a graduated cylinder, 20ml of 3% hydrogen peroxide solution to the conical flask (D) and 5 ml to the trap (E). Assemble the apparatus and connect condenser. Weigh 50g of blended sample into the round-bottomed flask (B) through gas inlet tube joint, using 300ml of water. Replace the inlet tube immediately, making sure all connections are well greased and tight. Remove the inlet tube, and slowly add 20ml of 4N HCl. Ensure that bubbles of nitrogen gas enter the receiving flask through the gas inlet tube. If not, check joints for leaks. Adjust nitrogen to give a flow of 15 to 20 bubbles per minute through the tube. Heat the content of the flask to boil and adjust the heater to give a slow boil. Continue

boiling for 30 mins. Stop heating and disconnect the assembly and remove the conical flask and the trap containing hydrogen peroxide. Transfer the hydrogen peroxide solution from the trap into the conical flask and rinse the trap with water and transfer the rinsing to the flask.

**Determination**

Add 3 drops of the indicator. Immediately titrate contents of conical flask (D) with 0.05N NaOH to yellow end point that persists for about 20 seconds. Compute sulfite content, expressed in mg $SO_2$/Kg food (ppm).

### 4.2.4 Observations

Weight of the sample = W = —— g

Normality of the NaOH solution = N

Volume of NaOH (titre) = V = —— ml

### 4.2.5 Calculations

1 ml of 0.05N NaOH = 1.6 mg of $SO_2$

Therefore, V ml of N normal NaOH = $\frac{(V \times N \times 1.6)}{0.05}$ = $(V \times N \times 32)$ — mg $SO_2$

Since $(V \times N \times 32)$ mg $SO_2$ is present in W g of the sample

Therefore, $SO_2$ (mg) in 1 Kg of the sample (ppm) = $\frac{(V \times N \times 32)}{W} \times 1000$

### 4.2.6 Result

$SO_2$ in the sample = ppm = mg per kilogram.

## 4.3 PRECAUTIONS

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

Handle the all glass distillation apparatus very carefully. It may easily break because the joints are rigid.