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

Structure

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°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 × V µg

C × 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.