DETERMINATION OF PHOSPHOLIPID CONTENT

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INTRODUCTION

Phospholipids are hydrolyzed by the action of phospholipase. The action of phospholipase is usually stronger than that of lipase. Therefore the extent of hydrolysis of phospholipids is adopted as an index of lipid deterioration.

Phospholipid content is obtained by using column chromatography to separate the triglycerides (neutral lipids) from the phospholipids. The polar phospholipids are absorbed by the silicic acid and eluted by the methanol. The neutral lipids which are not absorbed by the silicic acid are first eluted out by the chloroform.

APPARATUS

1. Glass chromatograph column (ø : 1 - 2 cm; length : 30 cm) with Teflon tap
2. Cotton wool
3. Filter paper (Whatman No. 1)
4. Preweighed, dry evaporating flask (50 ml capacity)
5. Analytical balance
6. Desiccator
7. Rotary evaporator with water bath (28°C)

REAGENTS

1. Silicic acid (Mallinchrodt, 100 mesh)
2. Celite 545
3. Methanol (analytical grade)
4. Chloroform (analytical grade)

PROCEDURE

Preparation of the packing material

1. Wash the silicic acid and Celite 545 separately with warm methanol for 5 to 10 mins.
2. Allow the material to settle and decant the washing solution.
3. Repeat steps 1 and 2 twice.
4. Wash with warm acetone twice.
5. Air dry at room temperature overnight.

6. Then oven dry at 120°C for 2 hours.

7. Cool and mix silicic acid and Celite 545 in a ratio 2:1

Preparation of column

1. Weigh packing material 10 - 15 times that of the lipid sample weight.

2. Soak cotton wool in chloroform and pack into bottom of column. Exclude as much air as possible.

3. Place 2 layers of Whatman No. 1 filter paper cut into size of columns.

4. Mix the packing material in chloroform and pour gently into column with the aid of a glass rod.

5. Allow the packing material to settle.

6. Place 2 layers of Whatman No. 1 filter paper on the packing material.

7. Drain column of excess chloroform leaving a 1 cm high column of chloroform.

ANALYTICAL PROCEDURE

1. Dissolve 1 g sample lipid in pure chloroform, making a 5 to 10% solution.

2. Introduce thin sample onto the column.

3. Drain off excess chloroform till solvent level is about 1 cm above the packing material.

4. Drain off with 250 ml chloroform and collect the neutral lipids in preweighed evaporating flask (elution speed : 3 drops per second).

5. Evaporate off the solvent using the rotary evaporator, dry up using a rotary vacuum pump for 5 mins, cool in desiccator for another 30 min and weigh the neutral lipids.

6. Drain off with 100 ml methanol and collect the phospholipids in preweighed evaporating flask.

7. Evaporate off the solvent using the rotary evaporator, dry up using a rotary vacuum pump for 5 mins, cool in desiccator for 30 min and weigh the phospholipids.

CALCULATION

$$\text{Neutral lipid (\%)} = \frac{\text{(Weight of Neutral Lipid)}}{\text{(Weight of PL + Wt. of NL)}} \times 100$$

$$\text{Phospholipid (\%)} = \frac{\text{(Weight of Phospholipid)}}{\text{(Weight of PL + Wt. of NL)}} \times 100$$

NL = Neutral lipid

PL = Phospholipid

The phospholipid content is expressed as the percentage of phospholipid over the total lipid present per gram of sample lipid.