EXTRACTION OF LIPIDS (MODIFIED FOLCH'S METHOD)

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INTRODUCTION

A mixture of chloroform and methanol in the ratio of 2:1 (v/v) extracts lipid more exhaustively from animal tissues than most other simple solvents systems. With most tissues, the lipids are removed almost completely after two or three treatments with the mixture. Most of the contaminating compounds in the extract can be removed from the chloroform-methanol (2:1 v/v) mixtures simply by shaking the combined solvents with a quarter of the total volume of water. The lower phase which comprises 60% of the total volume contains the purified lipid. This extraction yields approximately a 95 - 99% recovery of lipids.

APPARATUS

1. Homogenizer with ice jacket
2. Buchner flask and funnel
3. Vacuum pump
4. Nitrogen gas
5. Separating flasks (1000 ml)
6. Volumetric flasks (50 ml)
7. Measuring cylinders (100 and 250 ml)
8. Whatman No. 1 filter paper (qualitative, 7 cm ø)

REAGENTS

1) Purified and distilled chloroform

   Wash chloroform once with concentrated sulphuric acid (10 ml H_2SO_4 for 1 litre of chloroform). Then wash 2 to 3 times with distilled water using a separating funnel. Collect washed chloroform and add anhydrous calcium chloride. Stand overnight, then transfer to distillation flask. Distil and collect fraction which distills over at 60.5°C. Add purified and distilled methyl alcohol (1% by volume) as stabilizer. Keep in dark. Should be used within one month.

2) Purified and distilled methyl alcohol

   Add granular potassium hydroxide to methyl alcohol to remove acids, aldehydes and moisture. Distill and collect fraction which distills over at 64.5°C. Keep in the dark. Should be used within one month.

3) Chloroform-methyl alcohol (C-M mixture)

   Mix reagent from 1) with 2) in the proportion of 2:1 (v/v).

4) 1% BHA-BHT antioxidant solution

Dissolve 1 g of butylated hydroxyanisole (BHA) and 1 g of butylated hydroxytoluene (BHT) in 100 ml C-M mixture.

5) Anhydrous sodium sulphate

PROCEDURE

Sample Preparation

1. The fish sample is chopped into a mince. Depending on the tissue, the following approximate sample sizes are used.

   i) ordinary muscle (20 - 50 g)

   ii) dark muscle (15 g)

   iii) skin (10 g)

2. Weigh the chopped sample into the homogenizer cup.

3. Add C-M mixture volume of about 3.5 times the weight of sample, and 2-3 drops of antioxidant solution.

4. Homogenize for 1 min and filter with Whatman No. 1 filter paper using a Buchner funnel and vacuum pump.

5. Transfer the residue into the cup and repeat homogenization twice.*

6. Transfer the combined filtrate into a separating flask.

7. Pour distilled water, volume approximately a quarter of that of the extract, into the separating flask.

8. Shake very gently 2-3 times, and stand overnight.**

9. Drain off the chloroform phase through a Whatman No. 1 filter paper into an Erlenmeyer flask containing about 2 - 5 g anhydrous sodium sulphate. Shake well and leave for about 5 min. Decant into an evaporating flask.

10. Wash the filter paper 2 - 3 times with C-M Mixture.

11. Concentrate the extract with a rotary evaporator under reduced pressure at 40°C (water-bath temperature).***

12. Dissolve the concentrated extract with C-M Mixture and transfer to 50 ml volumetric flask using a pipette.

13. Make up to the mark with C-M Mixture.

14. Flush with nitrogen gas and store at -20°C. This sample is used for other tests unless otherwise specified.

* Not necessary to add antioxidant solution.

** When mixture does not separate well, centrifuge at 8,000 rpm for 10 min.

*** Do not allow to evaporate to dryness.

N.B. Residual water vapour cannot be removed completely.

REFERENCE

Christie, W.W. (1982). In: Lipid analysis (2nd Ed.) Pergamon Press:22

Folch et al. (1951). Journal of Biological Chemistry, 191:833.