EXTRACTION AND DETERMINATION METHOD OF LEAN FISH LIPIDS

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INTRODUCTION

Ethyl ether extraction method has been used generally and widely as extraction and determination method of lipids in foods. Neutral lipids such as triglyceride and wax are mostly extracted by ethyl ether, but most polar lipids such as phospholipids and galactolipids are not extracted by ethyl ether without acidic digestion.

Polar lipids contents of raw fish and shellfish are usually 0.5 - 1.0%, with the seasonal variation and the difference from fish species being less than that of neutral lipids.

Warm water fish normally has very low level fat content of several percent, so that the ratio of polar lipids to total lipids is larger than that of cold water fish. The difference between ethyl ether extracted lipid content and chloroform-methanol mixture extracted lipid content is larger than that of cold water fish.

Otherwise in the case of extraction from lean fish lipids, chloroform-methanol 1:1 mixture is more effective than chloroform-methanol 2:1 mixture and the former uses less volume of solvents than the latter.

APPARATUS

1. Homogeniser
2. Buchner flask and funnel
3. Vacuum pump or aspirator
4. Vacuum distillator or vacuum rotary evaporator
5. Nitrogen gas
6. Separating flask (1L)
7. Measuring cylinders (100 ml & 250 ml)
8. Filter paper, Whatman No. 1.

REAGENTS

1. Purified and distilled chloroform ($CHCl_3$).

2. Purified and distilled methanol (CH_3OH).

3. Anhydrous sodium sulfate ($NaSO_4$), analytical grade.

SAMPLE PREPARATION

(Refer to Fig. 1)

The raw fish sample (moisture content about 80%, fat content a few %) is chopped into a mince. 100g of the minced sample is homogenised with chloroform-methanol mixture using a homogeniser or a blender.

PROCEDURE

(Refer to Fig. 1)

1. Weigh 100 g of the chopped sample into the homogeniser cup.
2. Add 100 ml of chloroform and 200 ml of methanol and then homogenise for 2 mins with a homogeniser.
3. Add 100 ml more of chloroform to the homogenate and homogenise for 30 secs.
4. Add 100 ml of distilled water to the homogenate and homogenise it again for 30 secs.
5. Filter the homogenate using a Buchner funnel and vacuum pump.
6. Transfer the residue into the cup and repeat homogenisation once more using 50-100 ml of chloroform. Then filter the homogenate with Buchner funnel.
7. Transfer the combined filtrate into a separating flask and stand about an hour.
8. Drain off the chloroform phase into a flask containing about 5g of anhydrous sodium sulphate.
9. Filter the chloroform solution into a vacuum evaporating flask.
10. Wash the flask and the filter paper 2-3 times with chloroform.
11. Evaporate the extract with a rotary evaporator under reduced pressure.
12. Dry in a vacuum desiccator and weigh the crude lipids.
13. Flush with nitrogen gas and store at -20°C. This sample is used for other tests such as determination of lipids composition, fatty acid composition, fatty indexes and deterioration of fat.

REFERENCES

Folch, J., Lees, M. and Stanley, G.H.S. (1957) : J. Biol. Chem., 226, 497.

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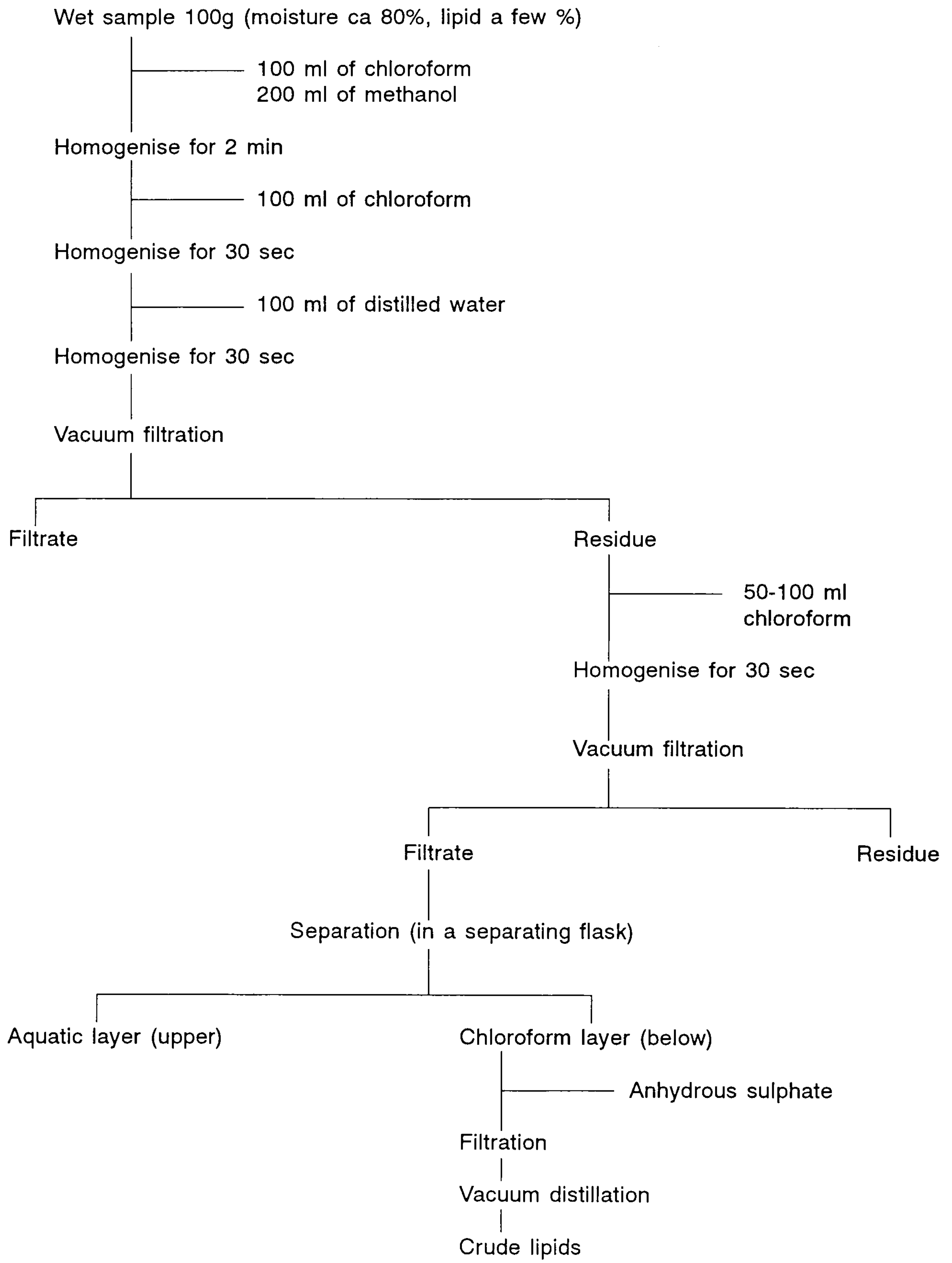

Fig. 1. Extraction Method of Lean Fish Lipids

(Taken from Bligh et. al (1959))