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INTERNATIONAL UNION OF PURE
AND APPLIED CHEMISTRY

APPLIED CHEMISTRY DIVISION

COMMISSION ON OILS, FATS
AND DERIVATIVES

STANDARD METHODS FOR THE ANALYSIS OF OILS, FATS AND DERIVATIVES

6th Edition, Part 1 (Sections I and II)

1st Supplement (1979)

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F O R E W O R D

In 1978 the Commission on Oils, Fats and Derivatives has revised all its previously published methods of anlaysis contained into the Sections I (Oleaginous seeds and fruits) and II (Oils and Fats). The corresponding book was recently published as the 6th Edition, Part 1, of the *"Standard Methods of Analysis of Oils, Fats and Derivatives"*, (Pergamon Press, Oxford, 1979).

During the Brussels meeting (August 1978) this Commission has adopted four new analytical methods. They are given here, as a first Supplement to the 6th Edition, Part 1.

C. P A Q U O T

Chairman of the Commission

1.123. DETERMINATION OF OIL CONTENT

(LOW RESOLUTION NUCLEAR MAGNETIC RESONANCE METHOD)

1. SCOPE

This Standard describes a quick method for the determination of oil content of oleaginous seeds and fruits.

2. FIELD OF APPLICATION

This Standard is applicable to oleaginous seeds and fruits giving an entirely liquid oil at the measuring temperature (basically 20°C).

It is not applicable, under current conditions of application, to seeds and fruits which do not give an entirely liquid oil at 20°C (shea nut, palm kernel, cocoa,...).

3. DEFINITION

In this Standard the oil is the whole of the organic substances contained in the oleaginous seeds and fruits which are liquid at the measuring temperature (basically 20°C).

4. PRINCIPLE

Determination by low resolution nuclear magnetic resonance (NMR) of liquid compounds containing hydrogen content in oleaginous seeds and fruits (mainly kernels and pips) which have been previously dehydrated by drying at 103 ± 2°C, applying a correction for the response of solids (cakes).

N.B. In the following text of this Standard the word "seeds" is used for seeds as well as kernels and pips.

5. APPARATUS

5.1. Continuous wave low resolution NMR spectrometer (Note 1)

5.2. Measuring tubes to fit the apparatus 5.1. and of maximum capacity, made of a non-conductive, non-magnetic material containing no hydrogen (e.g. glass, polytetrafluoroethylene)

5.3. Desiccator

6. REAGENTS

6.1. Crude oil from seeds of the same variety and origin as the seeds to be analysed, extracted according to method 1.122., less than one month in advance, and protected from autoxidation

6.2. Cake from seeds of the same variety and origin as the seeds to be analysed, freed from fat according to method 1.122. less than one month in advance and dried at 103 ± 2°C according to method 1.121.

7. PROCEDURE

7.1. Preparation of the Sample

Weigh exactly a quantity of seeds at least equal to 20 g and sufficient to fill a measuring tube (5.2.) and dry it according to method 1.121. (Note 2). Determine the water content.

Put each crystallizing dish containing the dry seeds into a desiccator (5.3.). The latter is placed very close to the NMR spectrometer at least several hours before the determination begins (Note 3) in a room where the temperature (generally about 20°C) does not undergo sudden fluctuations.

7.2. Calibration of the Spectrometer

Adjust the NMR spectrometer (5.1.) according to the instructions of the manufacturer, then adjust to zero after placing an empty measuring tube (5.2.) into the magnetic field.

Weigh, to within 0.010 g, 5, 10, 20, and 30 g (if this can be handled by the spectrometer) of a crude oil (6.1.) of the same variety and origin as the seeds to be analysed, into 4 tubes identical to the previous tube (i.e. giving a 0 reading when they are placed empty into the magnetic field) taking care that no droplet of oil adheres to the walls of the tube above its useful part.

Measure the spectrometer response for the 4 tubes. This gives R_5, R_{10}, R_{20} and R_{30}, as the mean values from 5 readings of the integrator for each tube for an integration time giving the best repeatability, applying the highest possible radiofrequency energy level without producing saturation (this result is obtained with most current spectrometers for an intensity of the detector collecting current between 100 and 150 microamperes).

Draw the straight line giving the values of R as a function of the oil weight in the tubes. This straight line must pass through the origin. If not, check the adjustment of the spectrometer.

7.3. Determination

Transfer the dehydrated seeds prepared according to 7.1. quantitatively and quickly into a measuring tube (5.2.) identical to the one used for the adjustment.

Without modifying the adjustment of the spectrometer, measure the response. This gives R, the mean value of this response from 5 readings of the integrator, using the previously chosen integration time.

7.4. Correction for the Cakes

Weigh exactly a sufficient amount of free from fat and dehydrated cake (6.2.) to fill a measuring tube (5.2.) identical to the one used for the adjustment.

Without modifying the adjustment of the spectrometer, measure the response. This gives C the mean value of this response from 5 readings of the integrator, using the previously chosen integration time.

8. EXPRESSION OF RESULTS

The apparent oil content in the seeds (o), as per cent (m/m), is given by the formula :

$$o = \frac{R \times m_x \times 100}{R_x \times m_o}$$

where :

m_o is the mass, in g, of the test portion before drying

m_x is the mass, in g, of the sample of x g of crude oil (20 or 30 g)

R is the response of the spectrometer for the test portion, as determined in 7.3.

R_x is the response of the spectrometer for x g of crude oil, as determined in 7.2. (R_{20} or R_{30})

The response of the cake (c), as per cent (m/m), in given by the formula :

$$c = \frac{C \times m_x \times 100}{R_x \times m'_o}$$

where :

m'_o is the mass, in g, of the free from fat and dehydrated cake used in 7.4.

C is the response of the spectrometer for m'_o g of free from fat and dehydrated cake, as determined in 7.4.

The oil content of the seeds, as per cent (m/m), is given by the formula :

$$\frac{o - c\,(1 - 0{,}01\,a)}{1 - 0.01\,c}$$

where :

a is the moisture content, as per cent (m/m), of the seeds, as determined according to method 1.121.

o and c are the previously calculated values.

9. NOTES

1 - The use of a low resolution pulse NMR spectrometer can be considered as an auxiliairy method only, not as a standard one. The dimensions and location of the measuring tubes allow only test portions of about 250 - 300 mg, i.e. measurements by the indirect method with a very small amount of seeds. Consequently it requires a large number of measurements to analyze at least 20 g of seeds. The accuracy of the mean value calculated from the results is generally insufficient.

2 - Trace amounts of ferrous metals alter the results. It is recommended to eliminate these with a magnet.

3 - It is important to recognize that a variation in temperature influences the results : the response of the oil varies by about - 0.3 per cent per degree. Consequently all calibrations and determinations must be made at exactly the same temperature. If the necessary equipment is not available, it is advisable to thermostate the "useful" part of the apparatus, and to place seed samples before determination into a metallic heat-insulated block which is kept at the measuring temperature.

II. OILS AND FATS 1979

2.503. DETERMINATION OF THE "OXIDIZED ACIDS"

(THIN LAYER CHROMATOGRAPHIC METHOD)

1. SCOPE

This Standard describes a method for the determination of the oxidized acids by thin layer chromatography.

2. FIELD OF APPLICATION

This Standard is applicable to non-heated animal and vegetable fats and oils.

3. DEFINITION

The oxidized fatty acids are the whole of the fatty acids containing one or several oxygenated functions, other than the carboxyl group, resulting from oxidizing attacks (or possibly being naturally present) and formed by monomeric, polymeric or partly degraded acids. In this Standard and by convention they are expressed as a percentage by mass of the ratio of ricinoleic acid to total fatty acids (without unsaponifiable matter).

4. PRINCIPLE

Preparation of the fatty acids without unsaponifiable matter. Separation by thin layer chromatography of non-oxidized acids and oxidized acids. Revelation by quantitative sulpho-chromic charring, then evaluation by photodensitometry of the intensity of the bands thus obtained. Conversion of the photodensitometric data into weight of carbon, then expression of the results in g of ricinoleic acid per 100 g of total fatty acids without unsaponifiable matter.

5. APPARATUS

5.1. Preparation of Fatty Acids and Chromatographic Separation

5.1.1. 250 ml ground-necked round-bottomed flasks
5.1.2. Refux tubes, to fit on the flasks 5.1.1.
5.1.3. 250 ml conical flask
5.1.4. 500 ml separating funnels
5.1.5. Funnel, with a long stem
5.1.6. 20 ml volumetric flask
5.1.7. Apparatus for distillation of solvents, ground-necked
5.1.8. Spreader for thin layer chromatography
5.1.9. Glass plates for thin layer chromatography, 5 x 20 cm (Note 1)
5.1.10. Glass developing tank for thin layer chromatography, with ground glass lid, preferably with channels
5.1.11. Ultramicroburette, or microsyringe with a hypodermic needle without pointed end (angle 90°), suitable to deliver, by 0.5 µl portions, volumes around 20 µl known to ± 0.1 µl, or manual or automatic apparatus suitable to deliver in a continuous manner the same volume with the same accuracy
5.1.12. Apparatus suitable to displace the plate in a discontinuous displacement by jumps of about 1 mm, or apparatus able to displace the plate in a continuous alternative manner (Note 2)
5.1.13. Boiling-water bath
5.1.14. Electric oven, regulable between 100 and 130°C
5.1.15. Pumice grains

5.2. Quantitative Interpretation by Photodensitometry

5.2.1. Sprayer giving a fine and homogeneous fog
5.2.2. Electric oven, regulable between 150 and 200°C
5.2.3. Recording photodensitometer

6. REAGENTS

6.1. Preparation of Fatty Acids and Chromatographic Separation

6.1.1. n-Hexane, dried over sodium sulphate or calcium chloride, then distillated over sodium
6.1.2. Diethyl ether
For chromatography, dry the ether over sodium sulphate or calcium chloride, then distil over sodium
6.1.3. Chloroform, dry
6.1.4. Glacial acetic acid
6.1.5. Solvent mixture for development : hexane (6.1.1.) / diethyl ether (6.1.2.) / acetic acid (6.1.4.) (60/40/1.5) (V/V/V)
6.1.6. Sodium sulphate, anhydrous
6.1.7. Silica powder, with binder (13 per cent) of the usual quality for chromatography
6.1.8. Potassium hydroxide, ethanolic solution about 2 N :
Dilute 120 ml of potassium hydroxide aqueous solution 8.5 N to 500 ml with 95 per cent ethanol (V/V)
6.1.9. Hydrochloric acid, 100 g/l aqueous solution
6.1.10. Sodium sulphate, 100 g/l aqueous solution

6.2. Quantitative Interpretation by Photodensitometry

6.2.1. Reference substance : pure methyl oleate, recently purified, or, failing this, mixture of methyl esters with high content of methyl oleate perfectly purified (e.g. methyl esters of olive oil) and having a molecular mass M exactly known (Note 3)
6.2.2. Potassium dichromate, saturated aqueous solution :
Dissolve 25 g of potassium dichromate in 100 ml boiling water, allow to stand overnight, decant or filter through glass wool
6.2.3. Sulphochromic mixture :
Pour cautiously, cooling to avoid losses, 85 g sulphuric acid 66°B into 10 ml water; the weight of this mixture shall not be less than 94 g. Cool to about 50°C, then add slowly, cooling frequently, 15 ml of the potassium dichromate solution (6.2.2.). Allow to cool to room temperature, then store at least 4 hours. Filter through glass wool.

7. PROCEDURE

7.1. Preparation of the Sample

Prepare the sample according to 2.001.

7.2. Preparation of the Fatty Acids

Weigh 5 g of the prepared sample into a flask (5.1.1.) and add 50 ml of the potassium hydroxide ethanolic solution (6.1.8.) and a pumice grain (5.1.15.). Fit the reflux tube (5.1.2.) and place the flask over the boiling-water bath (5.1.13.). Allow to boil for at least 20 min.

Cool, then transfer the contents of the flask into a separating funnel (5.1.4.). Add 150 ml of water.

Add 100 ml of diethyl ether (6.1.2.) and shake vigorously. Allow to settle, pour the lower hydroalcoholic phase into a second separating funnel (5.1.4.), then pour out the ethereal phase containing the unsaponifiable matter.

Repeat this extraction 3 times in the same manner (4 extractions altogether).

After the last separation, transfer the hydroalcoholic phase into a separating funnel (5.1.4.) containing already 50 ml of hydrochloric acid (6.1.9.). Stir thoroughly, then extract the fatty acids with 100 ml of diethyl ether.

Twice repeat this extraction of the aqueous phase by diethyl ether.

Combine the 3 extracts and wash them once with 50 ml of the sodium sulphate aqueous solution (6.1.10).

Extract this aqueous solution with 30 ml of diethyl ether and add the extract to the main ethereal fraction.

Dry rapidly (10 min) this ethereal solution over sodium sulphate (6.1.6.), filter through glass wool, evaporate the solvent in the apparatus (5.1.7.), dry to constant weight (about 30 min) in a flow of inert gas, under the vacuum of a water-jet pump, generally on a boiling-water bath, but not above 40°C if fatty acids of short or medium chain length are present.

7.3. Preparation of the Plates for Thin Layer Chromatography

Thoroughly clean the glass plates (5.1.9.) with ethanol, light petroleum and acetone in order to remove all fatty matter.

Put 30 g of the silica powder (6.1.7.) (calculated for 20 plates 5 x 20 cm) into a flask (5.1.3.). Add 60 ml of distilled water. Stopper and shake vigorously for 1 minute. Immediatly fill the slury into the spreader (5.1.8.). Spread a 0.25 mm thick layer on the cleaned plates.

Let the plates dry for 5 minutes in the air, then place them in the oven (5.1.14.) at 120°C for 1 hour for activation.

After cooling, wash the silica layers by letting the solvent mixture (6.1.5.) ascend through it in the developing tank (5.1.10.).

After a quick evaporation in the air, put the plates into the oven (5.1.14.) at 110°C overnight (Note 4).

After cooling the plates are stored protected from dust until use.

7.4. Chromatographic Separation

7.4.1. Preparation of the fatty acid solution

Weigh in a volumetric flask (5.1.6.), to within 0.5 mg, a suitable quantity of the fatty acids prepared in 7.2., make up to volume with chloroform (6.1.3.) and carefully homogenize.

The weight of the fatty acids must be such that the obtained chloroformic solution contains about 15 to 20 mg of oxidized acids. If the presumed content of the fat in oxidized acids is, or if an explorating preliminary determination gives a content of oxidized acids :
- lower than 0.5 per cent, weigh at least 3000 mg of fatty acids,
- between 0.5 and 1 per cent, weigh about 1700 mg,
- between 1 and 1.5 per cent, weigh about 1200 mg,
- between 1.5 and 2 per cent, weigh about 1000 mg,
- between 2 and 3 per cent, weigh about 600 mg,
- between 3 and 4 per cent, weigh about 400 mg,
- higher than 4 per cent, weigh not more than 200 mg.

In this last case, it is preferable to take a test portion of about 1000 mg and to dilute to 50, or even to 100 ml.

7.4.2. Preparation of the plates and deposits

Mark a fine line 5 mm away from, and parallel to, each edge of the plate by using a rule (e.g. template) and a pointed stirrer. Remove the silica powder from this line with a rigid edge of filter paper.

By means of the ultramicroburette (5.1.11.) and the apparatus (5.1.12.), and by collecting the droplets at the point of the needle, place an exacly known volume of about 20 ul of the chloroformic solution of the fatty acids at 1.5 cm above the bottom edge of the silica layer in a narrow and continuous band of about 20 mm length, parallel to the bottom edge and equidistant from the sides of the plate (Note 5). Prepare and load 8 plates per essay.

As soon as they are loaded, put the plates into the developing tank (5.1.10.) so as to keep them exactly vertical, separated from each other by about 1 cm, the first plate being also at 1 cm from the wall, and in a manner that the silica layer be not placed at more than 1 cm of a wall or the back of another plate.

7.4.3. Development of the plates

Pour into the developing tank, by help of a long-stemmed funnel (5.1.5.) and without touching the silica layers, 200 ml of the solvent mixture for development (6.1.5.). The upper level of the liquid must remain about 1 cm below the deposit line (Note 6).

Quickly put back the lid on the tank, develop by rising capillarity until the solvent front reaches a mark on the side of the silica layer at 10 cm above the deposit line.

Remove the plates and, after quickly drying in the air, put them for at least 30 min in the oven (5.1.14.) at 130°C (Note 4).

Cool the plates to room temperature.

7.5. Quantitative Determination by Photodensitometry

7.5.1. Revelation by quantitative charring

Spray the sulphochromic mixture (6.2.3.) homogeneously on the plates with the sprayer (5.2.1.). The sprayed mixture must be sufficient to moisten the silica layer totally without wetting it.

Quickly place the plates into the oven (5.2.2.) at 180°C for 1 hour (Note 7).

Remove the plates from the oven and allow to cool at room temperature. During the revelation the non-oxidized fatty acids have migrated to the front (important spot at a Rf of at least 0.80) and the oxidized fatty acids are distributed along several bands between the deposit line and the bottom of the non-oxidized band (Note 8).

This procedure of revelation by sulphochromic charring may possibly replaced by another procedure of revelation, provided this procedure gives quantitative results and especially provided the reference graph 7.5.3. be a straight line.

7.5.2. Photodensitometric measurement

By means of the recording photodensitometer (5.2.3.) measure by transmission the optical density of the bands formed by the oxidized fatty acids, taking for a reference the area of the layer below the deposit line and moved upwards until the bottom of the tailing of the non-oxidized acid band is reached.

On the chart trace the base line from the reference value before the spotting point to the bottom of the trail of the non-oxidized fatty acids.

Measure, preferably by planimetry, the area of the whole of the surfaces included between the base line and the recording curve from the starting point to the end of the last peak of the oxidized fatty acids.

From the spotted volume of the chloroformic solution, convert the measured area to an area corresponding to a volume of solution of exactly 20 µl. Calculate the mean of the corrected areas of at least 5 plates.

Convert into weight of carbon (in µg) by means of the reference graph 7.5.3.

7.5.3. Obtention of the reference graph

Dissolve 150 mg of the reference substance (6.2.1.) (preferably methyl oleate) in 100 ml of chloroform (6.1.3.). From this concentrated solution, prepare by dilutions, as described here, solutions in such a manner that by spotting of 20 or 30 µl of these solutions at the bottom of a plate deposits increasing from 1 to 12 µg of carbon are obtained.

	Concentrate solution (ml)	Final volume (ml)	Test portion (µl)	Content in C (µg)
Dilution I	10	20	20	11.55
Dilution II	5	20	30	8.67
Dilution III	10	50	30	6.96
"	"	"	20	4.64
Dilution IV	5	50	20	2.32
Dilution V	5	100	20	1.16

When using methyl esters of mean molecular mass M (close to 296) in place of methyl oleate, the contents of C mentioned in the last column must be multiplied by the factor 296/M.

Spot the reference substance on the plates prepared according to 7.3., and place them into the developing tank exactly as described in 7.4.2. Develop the plates on 10 cm height by rising capillarity with a mixture of hexane (6.1.1.) / diethyl ether (6.1.2.) (95/5) (V/V) (Note 9).

Reveal with the sulphochromic mixture as in 7.5.1., measure with the recording photodensitometer and evaluate the area of the obtained band as in 7.5.2.

Make at least 6 measurements for each point, calculate by linear regression the mean straight line with the weights of carbon (in µg) as abscissa and the planimetric areas as ordinate.

The reference straight line from areas corresponding to carbon masses between 1 and 12 µg for a single peak may be validly extrapolated to areas of 25 - 30 µg of carbon for practical determinations, where the measured area is the sum of several individual peaks.

7.5.4. Expression of results

The content of oxidized fatty acids, as per cent (m/m), is given by the formula :

$$\frac{138\ c}{m}$$

where :
c is the mass, in µg, of carbon calculated from the planimetric areas
m is the mass, in µg, of the test portion of fatty acids.

Carry out two determinations and take as the result the arithmetic mean of the two results.

Report the result to the first decimal place.

8. NOTES

1 - Plates of 10 x 20 cm or 20 x 20 cm may be used if they fit on the photodensitometer. In this case parallel bands of 5 cm width must be delimited on the layer by one or three lines traced with an agitator.

2 - A simple device for the discontinuous shifting of the plate is obtained by a moving reglet with a rack-and-pinion, the dents of which are spaced at 1 mm width and sliding between two guides, its displacements are commanded by a pinion provided with a milled button. The plate placed perpendicularly on the reglet can thus be regularly shifted below the tip of the needle of the microburette.

It is also possible, if no mechanical device is available, to shift the microburette by hand by using a guide-horse for marking the deposit line on which equal drops are spotted in regular intervals.

3 - If the methyl esters used for the determination of the reference graph do not give homogeneous band, purify them as follows : into a chromatographic column, 20 mm internal diameter and 300 mm height, filled with hexane, pour in a gentle spray 25 g of magnesium silico-alumi-

nate (e.g. Florisil). Allow to settle by gravity, then level the solvent to the upper surface of the column. Introduce 3 g of the product to be purified. Elute with 150 ml of a mixture hexane / diethyl ether (95/5) (V/V) and collect all the eluates. Evapore under inert atmosphere, preferably under reduced pressure, then dry to a constant weight.

4 - During the second drying in the oven, the acetic acid coming from the washing mixture and remaining on the silica layers must be entirely evaporated; this can be easily checked : no smell of acetic acid must remain on the plates.

To facilitate this evaporation of the acetic acid which is necessary in view to obtain light layers after charring, it is recommended to use a well-ventilated oven, preferably placed in a room free from solvent vapors.

5 - The correct length of the deposit band is a function of the photodensitometer cell dimension. It is necessary to determine for each apparatus the optimal length of this band and to have exactly the same lengths of deposits for the sample determinations and for the obtention of the reference straight line.

6 - With this solvent mixture and developing conditions, a mixture of equal parts of castor oil fatty acids and common fatty acids (peanut, olive, sunflower, soya) without unsaponifiable matter gives after charring two main bands with a Rf of 0.60 for the non-hydroxylated fatty acids and 0.30 for the ricinoleic acid.

If, owing to the quality of the silica gel, the temperature or any other factor, the measured Rf values are not close to these figures, the polarity of the solvent mixture must be modified in the right way by changing the hexane / diethyl ether ratio.

7 - It is recommanded to employ for the charring an oven distinct from the oven used for drying. The walls of this oven must be, if possible, resistant to the acidic vapors. This last recommendation is more important in the case of charring that in the case of drying.

8 - If all operations are carried out in the correct way, the plates show an almost perfectly white background and the bands are parallel and dark grey.

Eliminate plates showing an intensely grey background (poor preliminary washing - insufficient elimination of the acetic acid - pollution of the ovens), or reddened bands (insufficient charring), or bands which are not parallel (silica layers non homogeneous, or non-linear deposit).

9 - If, owing to the quality of the silica gel, the conditions of temperature or any other factor, the measured Rf value is not close to this figure, the polarity of the solvent mixture must be modified in the right way by changing the hexane / diethyl ether ratio.

II. OILS AND FATS 1979

2.641. DETERMINATION OF ORGANOCHLORINE PESTICIDES

1. SCOPE

This Standard describes a method for the determination of organochlorine pesticide content in oils and fats (Note 1).

2. FIELD OF APPLICATION

This Standard is applicable on the one hand to animal and vegetable oils and fats with the exception of fish oils, and to the other hand to organochlorine pesticides present at more than 0.01 to 0.05 p.p.m., and especially to the following compounds :

- hexachlorobenzene (HCB)
- α, β, γ, -hexachlorocyclohexane (HCH)
- 1,1-dichloro 2,2-bis(p-chlorophenyl)ethane (p,p-DDD or TDE)
- 1,1-dichloro 2,2-bis(p-chlorophenyl)ethylene (p,p-DDE)
- 1,1,1-trichloro 2,2-bis(p-chlorophenyl)ethane (p,p-DDT)
- 1,1,1-trichloro 2-(o-chlorophenyl) 2-(p-chlorophenyl)ethane (o,p-DDT)
- 1,1,1-trichloro 2,2-bis(p-methoxyphenyl)ethane (methoxychlor)
- heptachlor
- heptachlorepoxy
- endrin
- dieldrin
- aldrin.

This Standard is not applicable to waxes.

3. PRINCIPLE

Passing an hexanic solution of the fat over a micro-column of basic alumina of precisely defined activity. Gas-liquid chromatography of a portion of the eluate into an apparatus with an electron capture detector. Identification and determination of the organochlorine pesticides by comparison with reference mixtures.

4. APPARATUS

4.1. Chromatography columns, internal diameter 6 mm, height 95 mm, with an eluant reservoir of 15 ml minimal capacity, according to Fig. 1
4.2. 15 ml Calibrated conical tubes, with ground-necked stopper
4.3. Apparatus for solvent evaporation, e.g. micro-Snyder column
4.4. 10 and 250 ml Volumetric flasks
4.5. 0.5 and 1 ml Graduated pipettes
4.6. Gas-liquid chromatograph, with an electron capture detector, and column, internal diameter 2 mm, length 240 - 250 mm, filled with a stationary phase able to separate the various organochlorine pesticides (Note 2)
4.7. Silanized glass wool
4.8. 10 µl Syringe

5. REAGENTS

5.1. n-Hexane, for chromatographic analysis, nanograde purity
5.2. Benzene, for chromatographic analysis, nanograde purity
5.3. Solvent mixture : mixture of hexane (5.1.) / benzene (5.2.),(4 / 1) (V / V)
5.4. Alumina, basic, activity Super I, particle size 50-200 um (70-290 mesh) (Note 3)
5.5. Reference solution : weigh accurately to within 0.1 mg into a 250 ml volumetric flask (4.4.) :

HCB	25 mg
α-HCH	25 "
β-HCH	25 "
γ-HCH	25 "
heptachlor	50 "
aldrin	50 "
heptachlorepoxy	50 "
p,p'- DDE	100 "
dieldrin	50 "
o,p'- DDT	100 "
endrin	50 "
p,p'- DDD (TDE)	100 "
p,p'- DDT	150 "
methoxychlor	250 "

Adjust to the mark with benzene (5.2.). Transfer this solution into a ground-necked bottle and store in a refrigerator : solidification at low temperature of this solution will avoid losses through evaporation.

When needed, dilute this solution to 1/1000 with hexane (5.1.), or, better, with 2,2,4 trimethyl pentane (iso-octane) of the same purity, 5 µl of this last solution, when injected in the chromatograph, contains, in the order of elution of peaks, under the operating conditions so described in 6.4. :

HCB	0.5 ng
α-HCH	0.5 "
β-HCH	0.5 "
γ-HCH	0.5 "
heptachlor	1.0 "
aldrin	1.0 "
heptachlorepoxy	1.0 "
dieldrin	1.0 "
p,p'- DDE	2.0 "
o,p'- DDT	2.0 "
endrin	1.0 "
p,p'- DDD (TDE)	2.0 "
p,p'- DDT	3.0 "
methoxychlor	5.0 "

5.6. Internal standard solution : the internal standard should be an organochlorine pesticide not commonly encountered (Note 4).
For example prepare a 0.2 p.p.m. internal standard solution as follows : weigh accurately, to within 0.1 mg, 20 mg of the internal standard into a 10 ml volumetric flask (4.4.) and bring to volume with hexane (5.1.), and subsequently dilute this 4 times by a factor of 10. The final solution will now contain 1 ng of the internal standard per 5 µl.

5.7. Dye solution : dissolve 5 mg p-phenylazoaniline and 2.7 mg N,N-dimethyl-p-phenylazoaniline in 100 ml hexane (5.1.)

6. PROCEDURE

6.1. Deactivation of Alumina

Weigh the required quantity of alumina (5.4.) and transfer into a glass-stoppered bottle. Add quickly 10 per cent (m/m) of water (e.g. 5 g of water to 50 g of alumina), and close the bottle immediatly. Shake vigorously to break up lumps (the bottle will get warm during this procedure). Leave for 24 hours.

The deactivated alumina must be used within five days.

6.2. Activity Check for Alumina

Put a pad of silanized glass wool (4.7.) into the bottom of a chromatography column (4.1.). Weigh out 2.0 g of alumina deactivated according to 6.1. and transfer this into the column; Tap the walls of the column to secure a good packing.

With the aid of the graduated pipette (4.5.) transfer 0.5 ml of the dye solution (5.7.) into the column. Elute with 4.5 ml solvent mixture.

N,N-dimethyl-p-phenylazoaniline leaves an uncoloured band of 2 to 3 mm at the bottom of the column, and p-phenylazoaniline remains at the top of the column, leaving an uncoloured band of 1 to 2 mm. Fig. 2 shows the result which should be obtained.

If the activity found is too high (Note 5), adjust by adding the appropriate amount of water. Check again after 24 hours.

6.3. Separation of the Pesticides

Put a pad of silanized glass wool (4.7.) into the bottom of a chromatography column (4.1.). Weigh out 2.0 g of alumina deactivated according to 6.1. and corresponding to the activity check as described in 6.2., and transfer this into the column; tap the walls of the column to secure a good packing.

Weigh accurately, to within 0.1 mg, 0.4 to 0.5 g of the fat to be analysed into a 10 ml volumetric flask (4.4.), dissolve in a little hexane (5.1.) and bring to the mark with the same solvent. If only an identification is required, it is not necessary to weigh accurately.

Transfer 1 ml of this solution with the aid of the graduated pipette (4.5.) into the column. Rinse the walls of the column with 0.5 ml hexane. Then elute with 13 ml hexane, and collect all the eluates in a calibrated tube (4.2.). Concentrate to exactly 1 ml in the apparatus (4.3.).

For a quantitative determination, add, before concentration, exactly 1 ml of the internal standard solution (5.6.) with the aid of the graduated pipette (4.5.). The volume to be injected into the chromatographic column with the syringe (4.8.) is 5 µl and contains 1 ng of the internal standard.

6.4. Gas-Liquid Chromatography

Adopt the following conditions for the gas-liquid chromatography with the apparatus (4.6.)

Column temperature : 200 ± 5°C
Injector temperature : 225°C
Detector temperatures : ^{63}Ni : 300°C , Tritium : 215°C
Carrier gas : 20 - 25 ml N_2/min
Chart speed : 1.25 mm/min (0.5 inch/min)
Injected volume : 5 µl

7. EXPRESSION OF RESULTS

7.1. Qualitative Analysis

Compare the chromatogram obtained according to 6.4. with a chromatogram obtained with the reference solution (5.5.) under the same operating conditions and identify the constituents by means of their retention volume (or time), as compared with that of aldrin.

7.2. Quantitative Analysis

Having identified the pesticides contained in the sample according to 7.1., and taken in consideration the peaks heights, prepare a standard solution containing the known pesticides in a concentration near that in the sample, and add to this the internal standard (5.6.) in a ration of, e.g., 1 ng by 5 µl (Note 7). Then determine the height of (or the area under) the peaks from the pesticides present in the sample and compare with the height (or the area under) the peaks from the standard pesticides.

The content of pesticide *i* in parts per million (p.p.m.) (m/m) is given by the formula :

$$\frac{V_1 \times m_o \times h_i}{V_2 \times m \times h_o}$$

where :
m_o is the injected mass of the standard pesticide, in ng
m is the mass, in g, of the test portion
V_1 is the volume, in ml, of the test portion solution

V_2 is the injected volume, in µl (normally 5)

h_o is the peak height (or area) of the standard pesticide

h_i is the peak height (or area) of the pesticide i, measured with the same unit as h_o

8. NOTES

1 - In case of doubt, the Mills method, or the Nören and Westöö method, must be used as a reference method.

2 - The mixture of 3.15 per cent OV 17 and 3.85 per cent OV 210, on a support of granulometry 150 - 180 µm (80 - 100 mesh) is satisfactory.

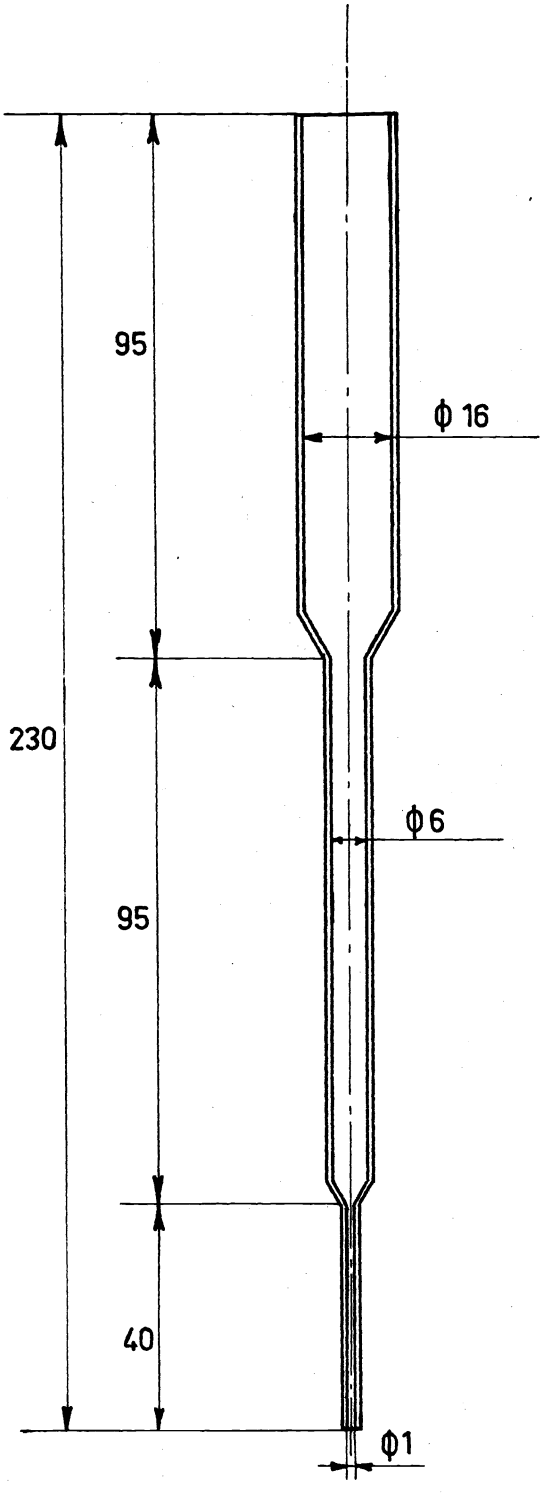
3 - Alumina W 200 basic WOELM, activity Super I, is satisfactory.

4 - Aldrin is normally satisfactory being rarely encountered.

5 - Deactivated alumina may increase in activity during storage. It is therefore advisable to prepare only the quantity required for a period of five days.

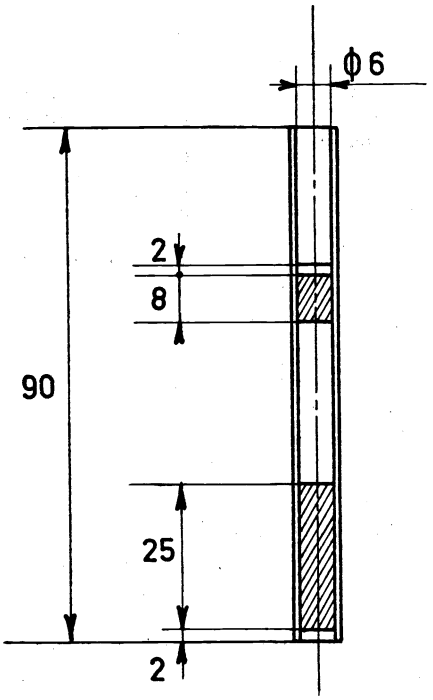
6 - If lower detection limits are required, double the test portion, use a chromatography column of double length and of same diameter, and double the quantities of reagents (alumina and hexane).

7 - The addition of the same quantity of the internal standard to the standard solution and to the extract from the test portion allows to verify the reproducibility of the injections and to apply a correction, if necessary, of peaks heights.



Dimensions in millimetres

Figure 1



Dimensions in millimetres

Figure 2

II. OILS AND FATS 1979

2.801. DETERMINATION OF TOTAL FAT IN MARGARINES

1. SCOPE

This Standard describes two methods for the determination of total fat in margarines : a quick method, and a method giving a simultaneous determination of the total fat content and of the non fatty components content.

2. FIELD OF APPLICATION

This Standard is applicable to all types of margarines, whatever their water content.

3. DEFINITIONS

The total fat in a margarine is the whole of the lipid components : triglycerides, mono and diglycerides, phospholipids, and possibly other liposoluble emulsifying agents.

The non-fatty components are the whole of the components other than total fat and water.

4. TOTAL FAT CONTENT (QUICK METHOD)

4.1. Principle

Adsorption of the water and retention of the fatty components by a sodium sulphate column. Elution of the fatty components by diethyl ether. Evaporation of the solvent, and weighing of the residue.

4.2. Apparatus

4.2.1. 250 ml Ground-necked round-bottomed flask
4.2.2. 100 ml Crystallizing dish (diameter 70 mm)
4.2.3. 250 or 500 Conical flask
4.2.4. Glass column, 26 mm internal diameter, 400 mm height, with a stopcock with a polytetrafluoroethylene plug
4.2.5. Apparatus for distillation of solvent, to fit on the flask 4.2.1.
4.2.6. Wire gauze scoop according to Fig, with 0.5 sq. mm mesh, 25 mm external diameter, 8 mm height, fitted on the side with a 50 - 100 mm vertical rod,- preferably in stainless steel (Note 1)
4.2.7. Desiccator
4.2.8. Electric oven, regulated at 103 $\pm$ 2°C
4.2.9. Boiling-water bath
4.2.10. Glass beads

4.3. Reagents

4.3.1. Diethyl ether, free from residue and peroxides, freshly distilled (Note 2)
4.3.2. Sodium sulphate, analytical reagent quality, anhydrous
4.3.3. Cotton wool, surgical quality, free from fat

4.4. Procedure

4.4.1. Preparation of the column

Put a small plug of the cotton wool (4.3.3.) at the bottom of the column (4.2.4.) with a glass rod.

Introduce into the column 100 ± 1 g of sodium sulphate (4.3.2.) in an even distribution.

Weigh 30 ± 1 g of sodium sulphate (4.3.2.) into the crystallizing dish (4.2.2.).

Remove the plug from the stopcock of the column (Note 3) and put the column and the crystallizing dish into the oven (4.2.8.) at 103 ± 2°C for 1 hour.

4.4.2. Sampling and test portion (Note 4)

With a piece of wire cut the block of margarine parallel to its three main axes into 8 parts. Retain two parts located diagonally. Put these two parts into a porcelain dish and carefully homogeneize them with a spatula.

From the homogeneized sample retain a 1.000 ± 0.050 g test portion. Put it into the center of the scoop (4.2.6.) previously tared. Determine the exact mass of the test portion.

4.4.3. Determination

Dry at 103 ± 2°C the flask (4.2.1.) containing 3 glass beads (4.2.10.), cool it in the desiccator (4.2.7.), tare and put it again into the desiccator until utilization.

Remove the column (4.2.4.) from the oven. Place the plug into the stopcock and close it. Fix the column vertically on a rack.

Introduce into the column the scoop (4.2.6.) containing the test portion and place it on the surface of the sodium sulphate, the temperature of which being then close to 100°C (Note 5)

Remove the crystallizing dish (4.2.2.) from the oven and recover the scoop with all the sodium sulphate contained in the crystallizing dish.

Wait until the column, or at least its external surface, has reached a temperature below about 35°C.

Then introduce cautiously 50 ml of diethyl ether (4.3.1.) in portions of 5 to 10 ml so as to produce as few bubbles as possible at the top of the column.

By little shocks or by rotation of the column, expell as many bubbles as possible from within the column.

Place a conical flask (4.2.3.) below the column.

Then open the stopcock of the column and let percolate 250 ml diethyl ether using 50 ml portions.

Collect all the eluates and transfer progressively into the flask (4.2.1.) containing 3 glass beads just removed from the desiccator. Fit the distillation apparatus (4.2.5.) to the flask and distill almost the totality of the solvent. Complete the evaporation of the solvent by heating in the boiling-water bath (4.2.9.) with the help of a slight flow of nitrogen.

Allow to cool in the desiccator. Weigh the residue to constant weight to the nearest 1 mg.

4.4.4. Blank test

Carry out simultaneously a blank test under the same conditions as the essay.

4.5. Expression of results

The content in total fat, as per cent (m/m), is given by the formula :

$$\frac{100\ (m_1 - m_2)}{m_o}$$

where :
m_o is the mass, in g, of the test portion
m_1 is the mass, in g, of the residue
m_2 is the mass, in g, of the residue of the blank test.

5. TOTAL FAT AND NOT-FATTY COMPONENTS CONTENT

5.1. Principle

Extraction of the total fat by light petroleum and retention of the water and the non-fatty components by pumice. Evaporation of the light petroleum and weighing of the residue (total fat). Drying of the pumice and determination of its weight increase (non-fatty components).

5.2. Apparatus

5.2.1. 250 ml Ground-necked round-bottomed flask, with a 24/29 female ground joint
5.2.2. Extraction tube, with a 24/29 male lower ground joint and a 45/40 female upper ground joint
5.2.3. Double grounded reducer, 45/40 male, 24/29 female
5.2.4. Reflux condenser, with a 24/29 male grounded joint
5.2.5. Apparatus for distilling solvents, to fit on the flask 5.2.1.
5.2.6. Weighing bottle, 50 x 90 mm, with a fitted grounded lid
5.2.7. Vacuum desiccator, with silica gel
5.2.8. Filtering crucibles, called Gooch, 35 mm diameter
5.2.9. Paper filter extraction thimbles, 27 x 30 mm, obtained, if necessary, by cutting a thimble of the same diameter but greater length
5.2.10. Boiling-water bath
5.2.11. Electric oven, regulated at 103 ± 2°C
5.2.12. Vacuum oven, regulated at 103 ± 2°C

5.3. Reagents

5.3.1. Light petroleum, b.p. 40 - 60°C, free from residue
5.3.2. Cotton wool, surgical quality, carefully free from residue
5.3.3. 1.5 - 2 mm Pumice stone

5.4. Procedure

5.4.1. Preparation of the extraction crucibles

In a filtering crucible (5.2.8.) put an extraction thimble (5.2.9.) containing about 2.5 g pumice (5.3.3.) covered with a small piece of cotton wool (5.3.2.).

Place all of it into the extraction tube (5.2.2.). Fit it on a flask (5.2.1.) containing 100 ml light petroleum (5.3.1.). Fit the reducer (5.2.3.) on the top of the extraction tube, then the condenser (5.2.4.). Extract by reflux for 2 hours.

From the extraction tube remove the crucible with its contents and put it into a weighing bottle (5.2.6.) without putting the lid on. Put the open weighing bottle, and its lid, into a vacuum oven (5.2.12.) regulated at 103 ± 2°C. Keep the oven open for 10 min, then close the

door and gradualy decrease the pressure to 10 torrs. Maintain 103 ± 2°C and 10 torrs for 50 min, then slowly bring back to atmospheric pressure and open the oven.

The complete weighing bottle / crucible / thimble, and the lid, are kept under vacuum in a desiccator (5.2.7.) containing silica gel (Note 6).

5.4.2. Sampling and test portion (Note 4)

Cut the block of margarine to be analysed with a piece of wire parallel to its three principal axes into 8 pieces. Retain two diagonally located parts. Put these two parts into a porcelain dish and carefully homogeneize with a spatula.

From the desiccator remove the assembly consisting of weighing bottle, crucible and thimble, fit the lid and determine the mass of the assembly.

Remove the crucible from the weighing bottle, remove the piece of cotton wool from the thimble, put it into the weighing bottle and close the bottle.

Take a test portion of 5.000 ± 0.050 g from the carefully homogeneize sample. Put it into the thimble above the pumice, then put the thimble into the crucible and the crucible into the weighing bottle, including the test portion.

Tare a flask (5.2.1.).

5.4.3. Extraction

Place the crucible and its contents on the neck of the tared flask. Introduce it together into an electric oven (5.2.11.) regulated at 103 ± 2°C and allow to remain for 2 hours. During this time a great part of the fat melts and flows into the flask.

Remove the flask and the crucible from the oven, and allow to cool.

Put 100 ml light petroleum (5.3.1.) into the flask. Gently cover the thimble with the piece of cotton wool, and put it together into the extraction tube (5.2.2.). Fit this extraction tube on the flask containing light petroleum, then the reducer (5.2.3.) and the condenser (5.2.4.) on the top of the extraction tube. Extract by reflux for 2 1/2 hours.

Remove the flask from the extraction tube and fit on the distillation apparatus (5.2.5.). Evaporate almost the totality of the solvent. Complete the evaporation of the solvent by heating in the boiling-water bath (5.2.10.)with the help of a slight flow of nitrogen.

Remove the crucible from the extraction tube and introduce it into the open weighing bottle. Put this into an electric oven (5.2.11.) regulated at 103 ± 2°C until an almost complete drying.

Then introduce both the weighing bottle and its contents, and the flask, into the vacuum oven regulated at 103 ± 2°C. Keep the oven open for 20 min, then close the door and gradually decrease the pressure to 10 torrs. Maintain 103 ± 2°C and 10 torrs for 50 min, then slowly bring back to atmospheric pressure and open the door.

Quickly put both the weighing bottle with its contents, and the flask, into a vacuum desiccator (5.2.7.) containing silica gel. After cooling, weigh these.

5.5. Expression of Results

The total fat content, as per cent (m/m), is given by the formula :

$$\frac{100 \times (m_3 - m_1)}{m_o}$$

The non fatty component content, as per cent (m/m), is given by the formula :

$$\frac{100 \times (m_4 - m_2)}{m_o}$$

where :
m_o is the mass, in g, of the test portion

m_1 is the mass, in g, of the weighing bottle including its lid, crucible, thimble with pumice and cotton wool

m_2 is the mass, in g, of the flask

m_3 is the mass, in g, of m_1 plus residue

m_4 is the mass, in g, of the flask and its residue

6. NOTES

1 - The wire gauze scoop is made by forming by hand around a metallic bar of about 23 mm diameter which is then removed. For ease to manipulation a metallic rod of 1 to 2 mm diameter and 50 to 100 mm length is brazed to the side of the gauze (see Figure).

2 - If the use of diethyl ether is prohibited for safety reasons, it may be replaced without problem by dichloromethane, analytical reagent quality, freshly distilled.

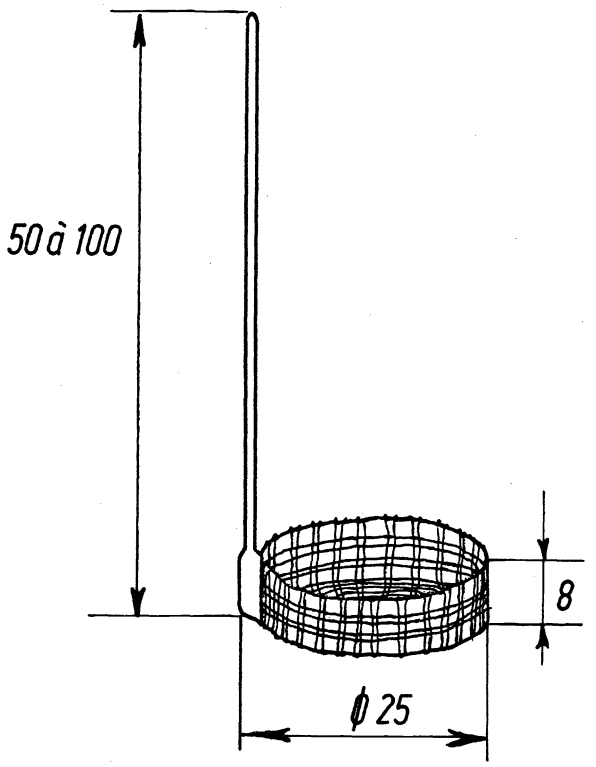
3 - The plugs of the polytetrafluoroethylene stopcocks frequently have parts made of other plastics (e.g. polypropylene); therefore it is recommended to avoid prolonged heating.

4 - The sampling method given is adapted to the determination of the true extemporary content in total fat of a block of margarine taken at any place at a given time.

If the aim is to ascertain whether the margarin conforms to the standards, disregarding superficial losses in water due to aging, it is necessary to remove from all sides of the margarine block a 0.5 to 1 cm layer, then to proceed on the central part as described previously.

5 - When the margarine is placed on the hot sodium sulphate, it melts almost instantaneously and sets free the emulsified water. This water is fixed by the adjacent sodium sulphate particles which rehydrate and agglomerate. It is preferable that the crust formed should not touch the column wall, because, in this case, the desorption of the fatty phase risks to be incomplete.

6 - The weighing bottle must kept open with its lid close by, as long as it is under reduced pressure. As soon as the atmospheric pressure is restored, the lid must be carefully fitted on the weighing bottle.



Dimensions in millimetres