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**STANDARD METHODS FOR THE
ANALYSIS OF OILS, FATS AND
DERIVATIVES**

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SECTION II: OILS AND FATS

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2.404. IDENTIFICATION AND DETERMINATION OF TOCOPHEROLS

I. SCOPE

This Standard describes a method for the separation of the various components of the tocopherol fraction present in fats and oils, namely tocopherols, tocopherol dimers, tocotrienols and tocotrienol dimers, and three methods for their determination: a colorimetric method (Method A), a gas-chromatographic method with a packed column (Method B) and a gas-chromatographic method with a capillary column (Method C).

2. FIELD OF APPLICATION

This Standard is applicable to animal and vegetable fats and oils with the following limitations:

Method A is suitable for the determination of tocopherol dimers and is applicable to all types of oils, including virgin olive oils.

Methods B and C are not applicable to virgin olive oils.

Methods A and B cannot be used to separate β and γ tocopherols which are determined together, nor the corresponding tocotrienols.

Method C is suitable for the determination of the four tocopherols and the four tocotrienols.

3. PRINCIPLE

Extraction of the total unsaponifiable matter under conditions which prevent oxidation. Fractionation of the total unsaponifiable matter by thin-layer chromatography and recovery of the tocopherol fraction or of its various components.

Method A: Reaction of each component with the mixture ferric-chloride - 2,2'-dipyridyl (Emmerie-Engel method). Determination of the absorbance at 520 nm and calculation with the aid of correcting factors.

Methods B and C: Gas-liquid chromatography on a non-polar phase and interpretation of the chromatograms; use of an internal standard for quantitative determinations.

4. APPARATUS

- 4.1. 50 ml and 100 ml ground-necked round bottom flasks
- 4.2. 15 ml pear-shaped flasks
- 4.3. 1 m long air-condenser to fit the 50 ml flasks (4.1.), or reflux condenser
- 4.4. 125 ml separating funnels
- 4.5. 1 ml volumetric pipette
- 4.6. 100 μ l graduated microsyringe
- 4.7. Glass plates for thin-layer chromatography, 20 x 20 cm, coated with silica gel, 0.25 mm thickness
- 4.8. Glass guard plates, 20 x 16 cm

2.404. page 2

- 4.9. Glass developing tank for thin-layer chromatography, with ground-glass lid, suitable for the 20 x 20 cm plates
- 4.10. Spray for thin-layer chromatography
- 4.11. Glass elution column, internal diameter 6 mm, height 95 mm, with an upper tube of 16 mm diameter and 95 mm in length, and with a lower capillary tube of 40 mm in length, as shown in Fig 1
- 4.12. Rotary evaporator, or failing this, boiling-water bath
- 4.13. Oven, regulated at $103 \pm 2^\circ\text{C}$
- 4.14. Clips for securing the guard plates (4.8.) to the plates (4.7.)

and for Method A :

- 4.15. 5 ml volumetric pipette
- 4.16. Spectrophotometer for reading in the visible spectrum
- 4.17. Cells, 1.00 ± 0.01 cm in width

and for Methods B and C :

- 4.18. 10 ml round bottom flasks, with ground joints
- 4.19. 1 ml graduated pipette
- 4.20. 1 μl microsyringe
- 4.21. Gas-liquid chromatograph, with flame-ionisation detector, and recorder
- 4.22. Glass column, about 2 m in length, internal diameter 2.2 - 2.5 mm, filled with 3 - 5 % of a methylpolysiloxane (SE 30 is suitable) or a phenylmethylpolysiloxane (OV 17 is suitable) on diatomaceous earth, particle size 150 - 180 μm (80 - 100 mesh) of quality suitable for gas-liquid chromatography, acid washed and silanised before use (for Method B)
- 4.23. Capillary glass column, about 20 m in length, internal diameter 0.4 mm, coated with a methylpolysiloxane (SE 30 is suitable) or a phenylmethylpolysiloxane (OV 17 is suitable) (for Method C)

5. REAGENTS

- 5.1. n-Hexane, for chromatography
- 5.2. n-Heptane, for chromatography
- 5.3. Benzene, anhydrous (maximum water content : 0.01 %)
- 5.4. Ethanol, absolute and free from aldehydes, analytical reagent quality (Note 1)
- 5.5. Diethyl ether, free from peroxides and residue (Note 2)
- 5.6. Developing solvent : mixture of hexane (5.1.) and diethyl ether (5.5.) 70:30 (v/v)
- 5.7. Elution solvent mixture : heptane (5.2.) and absolute ethanol (5.4.) 2:1 (v/v)
- 5.8. Pyrogallol, 5 g/100 ml ethanolic solution, prepared immediately before use (Note 3)
- 5.9. Potassium hydroxide, 1600 g/l aqueous solution. Prepare immediately before use 10 ml of this solution
- 5.10. Ferric chloride, ethanolic solution :
Dissolve 200 mg of ferric chloride ($\text{FeCl}_3 \cdot 6 \text{H}_2\text{O}$), analytical reagent quality, in 100 ml of absolute ethanol (5.4.). This solution must be prepared immediately before use
- 5.11. 2,2'-dipyridyl, ethanolic solution :
Dissolve 200 mg of 2,2'-dipyridyl in 100 ml of absolute ethanol (5.4.)
- 5.12. Reference solution of tocopherols :
Dissolve about 200 mg of a mixture in equal parts of the three tocopherols α , γ and δ in 10 ml of heptane (5.2.). In the absence of standard tocopherols, use the solution of the tocopherols prepared according to 6.1. and 6.2. from a mixture in equal parts of soyabean oil and sunflower oil. This solution gives a mixture in approximately equal parts of the three tocopherols

2.404, page 3

- 5.13. Phenolphthalein, 10 g/l solution in absolute ethanol (5.4.)
5.14. Nitrogen, containing less than 5 mg/kg of oxygen

and for Methods B and C :

- 5.15. Pyridine, analytical reagent quality, recently distilled over sodium hydroxide or barium oxide
5.16. Hexamethyldisilazane, pure
5.17. Trimethylchlorosilane, pure
5.18. Squalane, pure
5.19. Squalane (5.18.), 100 mg/100 ml solution in heptane (5.2.)
5.20. d-1 α tocopherol, purity not less than 99 %

6. PROCEDURE FOR THE SEPARATION OF THE TOCOPHEROL FRACTION

6.1. Extraction of the Total Unaponifiable Matter

Weight, to the nearest 0.005 g, about 1 g of the oil or fat to be analysed into a 50 ml flask (4.1.). Add 4 ml of the pyrogallol ethanolic solution (5.8.). Attach the air-condenser (4.3.) and bring to the boil. When the boiling starts, add 1 ml of the potassium hydroxide solution (5.9.). Boil for 3 min. Stop heating, cool the flask under running water, then add 25 ml of distilled water.

Transfer the contents of the flask quantitatively to a separating funnel (4.4.). Rinse the flask with 40 ml of diethyl ether (5.5.), transferring the rinsings to the separating funnel. Make a first extraction. Make two further extractions of the aqueous layer with 25 ml of diethyl ether each time, combining the three extractions in a second separating funnel. During every extraction, turn the separating funnel around its axis, without shaking, so that the formation of emulsions is avoided. Allow the layers to separate, then draw off the aqueous layer.

Wash the three combined extracts with 20 ml portions of water with vigorous shaking until the washing liquid does not turn pink on the addition of a drop of the phenolphthalein solution (5.13.) (Note 4).

Transfer the ethereal layer into a 100 ml flask (4.1.) and evaporate the diethyl ether by distillation using a rotary evaporator (4.12.) or, failing this, on a boiling-water bath. In order to dry the residue, add 1 ml of ethanol (5.4.) and 4 ml of benzene (5.3.) and evaporate under a stream of nitrogen (5.13.). Repeat a second time the addition of ethanol and benzene and the distillation of these solvents.

Dissolve the residue in 1 ml of hexane (4.1.) and transfer quantitatively the solution to a 15 ml pear-shaped flask (4.2.) using a minimum of hexane rinsings. Evaporate completely the hexane under vacuum or a stream of nitrogen (5.14.). Expel the air from the flask with the aid of a stream of nitrogen and add exactly 1 ml of heptane (5.2.) with the aid of a volumetric pipette (4.5.). Chromatograph immediately the solution so obtained.

6.2. Thin-Layer Chromatography

NOTE : All the following operations must be carried out away from daylight in a room dimly illuminated with the aid of an electric lamp protected by a clear red paper.

Saturate the glass developing tank (4.9.) with the development solvent (5.6.), lining the internal walls of the tank with filter paper. First activate a thin-layer plate (4.7.) by heating it for 30 min in an oven at 110°C (4.12.), then apply with the aid of the microsyringe (4.6.) about 1 μ l of the reference solution of tocopherols (5.12.) 2 cm from each side and 1 cm from the bottom. Starting 6 cm from the left side and in a band 2 cm wide, apply in small drops with the aid of the microsyringe the unaponifiable matter solution obtained according to 6.1. The quantity to be applied should be between 25 and 75 μ l depending on the tocopherol content of the oil; normally the applied quantity is 50 μ l. This quantity must be exactly measured for a quantitative determination. If there are several samples to be analysed on the same plate apply the samples successively with a clear space of 2 cm between two adjacent zones.

Introduce immediately the plate into the developing tank previously saturated, fit the lid and develop away from light until the solvent front reaches 1 cm below the top of the plate. Remove the plate and immediately cover with the guard plate (4.8.) fixing it in position with two clips (4.14.), and leaving two 2 cm bands at the left and the right (see Fig 2). Spray

2.404, page 4

immediately with the spray (4.10.) the two uncovered zones of the silica with a mixture of equal volumes of the ferric chloride (5.10.) and 2,2'-dipyridyl (5.11.) solutions. Place the plate in the dark for 5 to 10 min. Deep pink spots will appear indicating the location of the tocopherols derived from the reference solution.

Remove the guard plate, and, with the spots already revealed as reference marks, trace onto the plates rectangles about 2 cm wide in which are located the tocopherols of the applied samples. Remove the silica contained in each of these rectangles, with the usual precautions for quantitative recoveries.

Transfer the silica so obtained from each rectangle to an elution column (4.11.), adding the silica in small portions at a time and facilitating the packing by gently tapping the sides of the column.

Place the packed column over a pear-shaped flask (4.2.). Extract the silica with 5 to 6 ml of elution solvent (5.7.). Evaporate the solvents under vacuum, then immediately proceed to the determination of the tocopherols in the residue according to one of the procedures outlined below.

7. COLORIMETRIC DETERMINATION OF THE TOCOPHEROLS (Method A)

7.1. Procedure

NOTE : All the following operations must be carried out in a dark room, dimly illuminated with a red light.

Add with the aid of the graduated pipette (4.15.) to each pear-shaped flask containing each tocopherol fraction obtained according to 6.2., 3.6 ml of ethanol (5.4.), then 0.2 ml of the 2,2'-dipyridyl solution (5.11.) and 0.2 ml of the ferric chloride solution (5.10.). Homogenise by small rotations of the flask, and allow to stand for 10 min.

Make a blank test under the same conditions with the same quantities of reagents, but without tocopherols.

Transfer the solutions to cells (4.17.), and, using the spectrophotometer (4.16.), measure the absorbance at 520 nm of the test solution and of the blank against ethanol (5.4.). The blank must give an absorbance not greater than 0.05.

7.2. Expression of Results

The content of each tocopherol, in mg/100 g, is given by the formula :

$$\frac{(A - A_0) \times F \times V \times 1000}{v \times m}$$

where :

A is the absorbance of the test solution

A₀ is the absorbance of the blank solution

V is the volume, in ml, of the heptane used to dissolve the unsaponifiable matter in 6.1.

v is the volume, in μ l, of the unsaponifiable matter solution applied to the plate in 6.2.

m is the mass, in g, of the test portion

F is a spectrophotometric factor different for each tocopherol, and equal to :

98 for α tocopherol

90 for β tocopherol and γ tocopherol

75 for δ tocopherol

8. GAS-LIQUID CHROMATOGRAPHY WITH PACKED COLUMN (Method B)

8.1. Preparation of the Silyl Ethers

Add to a flask (4.18.), with the aid of the graduated pipette (4.19.), in order : 0.5 ml of pyridine (5.15.), 0.45 ml of hexamethyldisilazane (5.16.) and 0.3 ml of trimethylchlorosilane

(5.17.). Close the flask and allow to stand for 15 min until total separation of the white precipitate occurs.

Transfer, with the aid of the syringe (4.6.), 100 μ l of the supernatant clear liquid to the pear-shaped flasks containing each tocopherol fraction obtained according to 6.2. The dissolving of the tocopherols is facilitated by inclination of the flask and by rotating it around its axis. Allow to stand for 5 min before proceeding to analyse the solutions by gas chromatography.

8.2. Determination of the Tocopherol Profile

8.2.1. Procedure

For the analysis by gas chromatography using the apparatus (4.21.) and the column (4.22.) the following conditions should be observed :

column temperature : 240°C
 injector temperature (if this is independent of the preceding) : 250°C
 detector temperature : 250°C
 carrier gas : 0.8 bar

Inject 0.5 - 1 μ l of the silanised solution obtained according to 8.1., which, depending on the chromatographic response, may be concentrated by evaporation under a flow of nitrogen.

8.2.2. Identification of the Tocopherols

Under these conditions and with the use of a methylpolysiloxane stationary phase (SE 30), the relative retention times with respect to α tocopherol are approximately :

α tocopherol	1.00
β + γ tocopherol	0.71
δ tocopherol	0.55

8.2.3. Expression of Results

Using the method of internal normalisation (assuming total elution), the relative percentage of each tocopherol is given by the formula :

$$\frac{A_i \times 100}{\sum A_i}$$

where :

A_i is the area under the peak corresponding to the tocopherol i

$\sum A_i$ is the sum of the areas under all the peaks corresponding to the tocopherols, expressed in the same units as A_i .

8.3. Quantitative Determination of the Tocopherols

8.3.1. Determination of the Reference Curve

Weight exactly in four flasks (4.18.) mixture of α tocopherol (5.20.) and squalane (5.18.) in the following proportions : squalane/ α tocopherol : 0.5/1, 1/1, 2/1 and 3/1.

Prepare the silyl ethers according to 8.1. and proceed to the chromatographic analysis according to 8.2.1. Plot the graph, taking the ratio weight of squalane/weight of α tocopherol as abscissae and the ratio area under the peak of squalane/area under the peak of α tocopherol as ordinates. This graph should be a straight line through the origin. Calculate from this straight line the ratio R :

$$R = \frac{\text{weight of squalane/weight of } \alpha \text{ tocopherol}}{\text{area under the peak of squalane/area under the peak of } \alpha \text{ tocopherol}}$$

2.404. page 6

8.3.2. Introduction of the Internal Standard

Add to the pear-shaped flasks containing each tocopherol fraction obtained according to 6.2., with the aid of the graduated pipette (4.19.) or the volumetric pipette (4.5.), 1 ml of the squalane solution (5.19.). Evaporate the solvent under vacuum and proceed immediately to the silanisation according to 8.1.

8.3.3. Procedure

Proceed exactly as in 8.2.1.

8.3.4. Identification of the Tocopherols

Under these conditions, and with the use of a methylpolysiloxane (SE 30) stationary phase, the relative retention times with respect to squalane are approximately :

α tocopherol	3.36
β + γ tocopherol	2.40
δ tocopherol	1.85
squalane	1.00

8.3.5. Expression of Results

The content of tocopherol i , in mg/100 g, is given by the formula :

$$\frac{R \times A_i \times 100,000}{A_s \times m \times v}$$

where :

v is the volume, in μ l, of the unsaponifiable matter solution applied to the plate in 6.2.

m is the mass, in g, of the test portion

A_i is the area under the peak corresponding to the tocopherol i

A_s is the area under the peak corresponding to the squalane, expressed in the same units as A_i

R is the calculated value peak according to 8.3.1.

9. GAS-LIQUID CHROMATOGRAPHY WITH CAPILLARY COLUMN (Method C)

9.1. Procedure

For the gas-liquid chromatography with the apparatus (5.21.) and the column (5.23.) the following conditions should be observed :

column temperature	: 240°C
injector temperature	: 250°C
detector temperature	: 250°C
carrier gas, column flow	: 2 ml/min
splitter vent flow	: 60 ml/min

Prepare the silyl ethers of the tocopherol fractions obtained according to 6.2. as described in 8.1. Evaporate the reagents by a flow of nitrogen. Dissolve the dry residue in 1 ml of diethyl ether (5.5.). Draw 1 μ l into the needle of the injector, allow the solvent to evaporate and inject.

9.2. Identification of the Tocopherols

Under these conditions, and with the use of a methylphenylpolysiloxane (OV 17) or a methylpolysiloxane (SE 30) stationary phase, the relative retention times with respect to α tocopherol are approximately :

2.404. page 7

	OV 17	SE 30
α tocopherol	1.00	1.00
β tocopherol	0.64	0.68
γ tocopherol	0.66	0.70
δ tocopherol	0.50	0.54
α tocotrienol	1.71	1.31
β tocotrienol	1.09	0.89
γ tocotrienol	1.13	0.92
δ tocotrienol	0.86	0.70

9.3. Quantitative Determination of the Tocopherols and Expression of Results

Introduce an internal standard as in 8.3.3., chromatography as in 9.1. and express the results as in 8.3.5.

10 NOTES

1 - If it is necessary to prepare absolute ethanol free from aldehydes, proceed as follows :

All the operations must be carried out in an all-glass apparatus.

Add 2 g of potassium hydroxide and 1 g of potassium permanganate per litre of commercial absolute ethanol, low in aldehydes (molasses ethanol, 99.5 % minimum). Boil under reflux for about 30 min, then distil. Dry over anhydrous calcium sulphate. Distil a second time with protection against the ingress of moisture.

2 - If it is necessary to prepare diethyl ether free from peroxides and residue, proceed as follows :

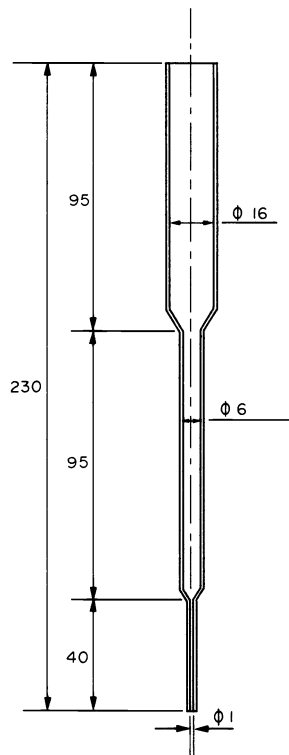
Transfer to a brown glass bottle 130 g of molecular sieve 4 Å beads of about 2 mm diameter for every litre of diethyl ether, analytical reagent quality. Shake vigorously and allow to settle for 12 h minimum in the closed bottle. Store the diethyl ether under the same conditions.

In order to eliminate peroxides, decant the dried diethyl ether and elute it through a column, 22 mm in diameter and about 600 mm in length, filled with 100 g of basic alumina, activity I, particle size 0.063 - 0.200 mm, with a flow of diethyl ether of about 2 - 3 ml/min. Collect the eluate in a brown glass bottle.

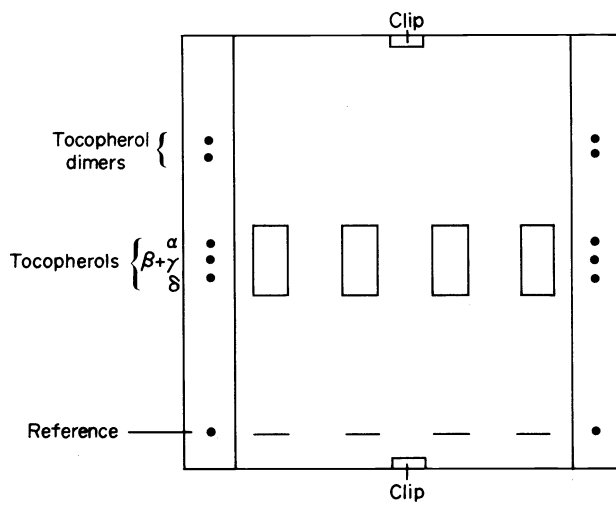
3 - The pyrogallol ethanolic solution may be replaced by a solution of 0.3 g of ascorbic acid in 4 ml of ethanol prepared immediately before use.

4 - During the washing with distilled water and principally during the first washing it is necessary to avoid shaking too vigorously in order to prevent the formation of emulsions. If the two layers do not separate after a few seconds, add a few ml of saturated aqueous sodium chloride solution to break the emulsion. The separation of the two layers should take place immediately.

2.404. page 8



Dimensions in millimetres

Figure 1Figure 2

II. OILS AND FATS

1981

2.507. DETERMINATION OF POLAR COMPOUNDS IN FRYING FATS

1. SCOPE

This Standard describes a method for the determination of polar compounds in frying fats. Polar compounds are formed during the heating of fats.

2. FIELD OF APPLICATION

This Standard is applicable to animal and vegetable oils and fats. The method serves to assess the deterioration of used frying fats.

3. DEFINITION

Polar compounds are those compounds in fats which are determined by column chromatography under the conditions specified (Note 1).

4. PRINCIPLE

Separation of frying fats by column chromatography into non-polar and polar compounds. Elution of the non-polar compounds. Determination of polar compounds by calculating the difference between the weight of the sample added to the column and that of the non-polar fraction eluted.

5. APPARATUS

- 5.1. 250 and 500 ml round bottom flasks with ground necks
- 5.2. 100 ml beakers
- 5.3. Ground-glass stoppers to fit the 500 ml flasks (5.1.)
- 5.4. Chromatographic glass column, 21 mm internal diameter, 450 mm in length, with stopcock (preferably in polytetrafluorethylene), and ground-glass joint
- 5.5. 250 ml dropping funnel, with ground-glass joint to fit the column (5.4.)
- 5.6. Glass funnel, about 8 cm diameter
- 5.7. Glass rod, about 60 cm in length
- 5.8. 50 ml volumetric flask
- 5.9. 20 ml volumetric pipette
- 5.10. 2 μ l capillary pipettes for thin-layer chromatography
- 5.11. Glass plates for thin-layer chromatography, 20 x 20 cm, coated with silica gel (without fluorescence indicator), 0.25 mm layer thickness
- 5.12. Glass developing tank for thin-layer chromatography, with ground-glass lid
- 5.13. Spray for thin-layer chromatography
- 5.14. Porcelain dish, about 20 cm diameter

2.507. page 2

- 5.15. Oven regulated at $103 \pm 2^{\circ}\text{C}$
- 5.16. Drying oven controllable between 120 and 160°C
- 5.17. Water bath
- 5.18. Desiccator, containing a suitable desiccant such as silica gel with moisture indicator (blue gel)
- 5.19. Apparatus for removing solvent under vacuum, e.g. rotary evaporator
- 5.20. Shaking machine

6. REAGENTS

- 6.1. Light petroleum (b.p. $40 - 60^{\circ}\text{C}$), chromatographic quality, redistilled
- 6.2. Ethanol, 95 % (v/v)
- 6.3. Chloroform, pure
- 6.4. Diethyl ether, free from peroxides and residue
- 6.5. Acetic acid, 100 %, analytical reagent quality
- 6.6. Elution solvent : mixture of light petroleum (6.1.) and diethyl ether (6.4.), 87/13, (V/V)
- 6.7. Developing solvent : mixture of light petroleum (6.1.), diethylether (6.4.) and acetic acid (6.5.), 70/30/2, (V/V/V)
- 6.8. Silica gel, particle size 0.063 - 0.200 mm (70 - 230 mesh), Merck N° 7734 or equivalent, adjusted to a water content of 5 % (m/m) (Note 2)
- 6.9. Phosphomolybdic acid, analytical reagent quality, 100 g/l solution in ethanol (6.2.)
- 6.10. Sea sand, purified by acid and calcined
- 6.11. Cotton wool, surgical quality
- 6.12. Nitrogen, 99.0 - 99.8 %

7. PROCEDURE

7.1. Preparation of the Sample

Remove visible impurities by filtration after homogenisation. If water is present, use a hydrophobic filter paper.

For semi-liquid and solid samples, warm to a temperature slightly above the melting point and homogenise carefully, avoid overheating.

7.2. Preparation of the Column

Fill the column (5.4.) with about 30 ml of the elution solvent (6.6.). Introduce a wad of cotton wool (6.11.) into the lower part of the column with the aid of a glass rod (5.7.) and remove air by pressing the wool.

Prepare in a beaker (5.2.) a slurry of 25 g of silica gel (6.8.) in about 80 ml of the elution solvent and pour this slurry into the column with the aid of the funnel (5.6.). To ensure complete transfer of the silica gel into the column, rinse with the elution solvent.

Open the stopcock and drain off the elution solvent into a second beaker (5.2.) until the level of the elution solvent is 10 cm above the silica gel. Level the silica gel by tapping against the column.

2.507. page 3

Add about 4 g of sea sand (6.10.) with the aid of the funnel. Drain off the supernatant elution solvent as far as the sand layer (Note 3).

7.3. Column Chromatography

NOTE : For the determination of polar compounds, the non-polar fraction is used only. However, if the efficiency of the fractionation is assessed by thin-layer chromatography or by recovery of the sample, the polar and non-polar fractions are required.

Weigh, to the nearest 0.001 g, 2.5 ± 0.1 g of the sample prepared as 7.1. into a volumetric flask (5.8.). Dissolve in about 20 ml of the elution solvent (6.6.) while warming slightly. Allow to cool to room temperature, and fill up to the mark with the elution solvent.

Introduce with a volumetric pipette (5.9.) 20 ml of this solution onto the column prepared as 7.2. Avoid disturbing of the surface (Note 4).

Dry two 250 ml flasks (5.1.) in the oven (5.15.) at a temperature of $103 \pm 2^\circ\text{C}$. Allow to cool to room temperature and weigh accurately to within 0.001 g. Place one of them under the outlet of the column.

Open the stopcock and let the sample solution drain off to the level of the sand layer.

Elute non-polar compounds with 150 ml of the elution solvent (6.6.) using a dropping funnel (5.5.). Adjust the flow rate so that 150 ml passes through the column within 60 - 70 min.

After completion of the elution, wash any substance adhering to the outlet of the column into the flask with the elution solvent using a pipette (5.9.).

If the polar compounds are required, elute them into a second 250 ml dry flask with 150 ml diethyl ether (6.4.) as described before.

After completing the elution, the silica gel is discarded.

Remove the solvent from the flask(s) with the aid of a rotary evaporator (5.19.) using a water bath (5.17.) at a temperature no higher than 60°C . Avoid losses due to foaming (Note 5).

Shortly before the end of the distillation, introduce nitrogen (6.12.) into the system.

Weigh the flask(s).

8. EXPRESSION OF RESULTS

The content of polar compounds, in per cent (m/m), is given by the formula :

$$\frac{m - m_1}{m} \times 100$$

where :

m_1 is the mass, in g, of the non-polar fraction

m is the mass, in g, of the sample contained in 20 ml of the solution added to the column

9. THIN-LAYER CHROMATOGRAPHIC ASSESSMENT OF COLUMN EFFICIENCY

The efficiency of the fractionation can be assessed by thin-layer chromatography (Note 6).

For the thin-layer chromatographic investigation prepare 10 % solutions of the substances in chloroform (6.3.), and apply 2 μl spots onto a plate (5.11.) using a capillary pipette (5.10.).

Line the developing tank (5.12.) with filter paper to achieve saturation. Place the plate in the developing tank and carry out the development with the developing solvent (6.7.). Normally, after 35 min, the solvent front ascends to a height of about 17 cm. Remove the plate and allow the solvent to evaporate.

Spray the plate with the phosphomolybdic acid solution (6.9.). After evaporation of ethanol, heat the plate in the drying oven (5.16.) at $120 - 130^\circ\text{C}$. As an example see Fig. showing a chromatogram obtained after fractionation of a frying fat into individual fractions.

2.507. page 4

10. NOTES

1 - The polar compounds include polar substances such as monoglycerides, diglycerides, free fatty acids which occur in unused fats as well as polar transformation products formed during frying of foodstuffs and/or during heating. Non-polar compounds are mostly unaltered triglycerides.

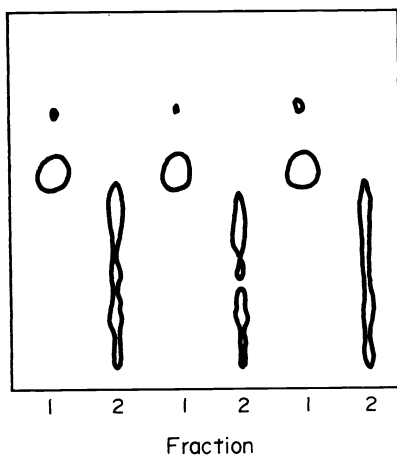
2 - Place the silica gel in the porcelain dish (5.14.), dry in an oven (5.16.) at 160°C for at least 4 h and cool in a desiccator (5.18.) to room temperature. Adjust the silica gel to a water content of 5 % : e.g. weigh 152 g of silica gel and 8 g of water in a 500 ml flask (5.1.). Close the flask with a stopper (5.3.) and shake mechanically with the aid of the shaking machine (5.20.).

3 - The excess solvent mixture drained off should not be used for elution.

4 - For fats containing low amounts of polar components the amount of sample added to the column can be raised from 1 up to 2 g.

5 - If a rotary evaporator is not available the elution solvent can be evaporated on a steam plate under a stream of nitrogen.

6 - The efficiency of the fractionation can also be assessed by checking the recovery of the sample. For samples containing greater amounts of polar material, recovery of the sample may be incomplete. This is due to small amounts of highly polar material, generally not more than 1 - 2 %, which is not eluted under the conditions specified.



1: Non-polar compounds
2: Polar compounds