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SECOND COMMISSION DIRECTIVE
of 18 November 1971
establishing Community methods of analysis for the official control of feedingstuffs

(71/393/EEC)

(OJ L 279, 20.12.1971, p. 7)

Amended by:

		Official Journal		
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►**M1**	Commission Directive 73/47/EEC of 5 December 1972	L 83	35	30.3.1973
►**M2**	Commission Directive 81/680/EEC of 30 July 1981	L 246	32	29.8.1981

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establishing Community methods of analysis for the official control of feedingstuffs

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THE COMMISSION OF THE EUROPEAN COMMUNITIES,

Having regard to the Treaty establishing the European Economic Community;

Having regard to the Council Directive of 20 July 1970 [1] on the introduction of Community methods of sampling and analysis for the official control of feedingstuffs, and in particular Article 2 thereof;

Whereas that Directive requires that official controls of feedingstuffs be carried out using Community methods of sampling and analysis for the purpose of checking compliance with requirements arising under the provisions laid down by law, regulation or administrative action concerning the quality and composition of feedingstuffs;

Whereas Commission Directive No 71/250/EEC of 15 June 1971 [2] has already established a number of Community methods of analysis; whereas the progress of work since then makes it advisable to adopt a second set of methods;

Whereas the measures provided for in this Directive are in accordance with the Opinion of the Standing Committee for Feedingstuffs;

HAS ADOPTED THIS DIRECTIVE:

Article 1

The Member States shall require that analyses for official controls of feedingstuffs as regards their contents of moisture, volatile nitrogenous bases, total phosphorus and crude oils and fats be carried out according to the methods described in the Annex to this Directive.

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Article 2

The Member States shall, not later than 1 January 1973, bring into force the laws, regulations or administrative provisions necessary to comply with this Directive. They shall forthwith inform the Commission thereof.

Article 3

This Directive is addressed to the Member States.

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[1] OJ No L 170, 3.8.1970, p. 2.
[2] OJ No L 155, 12.7.1971, p. 13.

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ANNEX

I. DETERMINATION OF MOISTURE

1. Purpose and Scope

This method makes it possible to determine the moisture content of feedingstuffs. ►M1 It does not cover the analysis of milk products as straight feedingstuffs, the analysis of mineral substances and mixtures composed predominantly of mineral substances, the analysis of animal and vegetable fats and oils or the analysis of the oil seeds and oleaginous fruit defined in Council Regulation No 136/66/EEC [1] of 22 September 1966 on the establishment of a common organization of the market in oils and fats. ◄

The determination of the moisture content of oil seeds and oleaginous fruit is described in Annex III to Commission Regulation (EEC) No 1470/68 [2] of 23 September 1968 on the drawing and reduction of samples and the determination of the oil content, impurities and moisture in oil seeds.

2. Principle

The sample is desiccated under specified conditions which vary according to the nature of the feedingstuff. The loss in mass is determined by weighing. It is necessary to carry out preliminary drying when dealing with solid feedingstuffs which have a high moisture content.

3. Apparatus

3.1. Crusher of non moisture-absorbing material which is easy to clean, allows rapid, even crushing without producing any appreciable heating, prevents contact with the outside air as far as possible and meets the requirements laid down in 4.1.1. and 4.1.2. (e.g. hammer or water-cooled micro-crushers, collapsible cone mills, slow motion or cog-wheeled crushers).

3.2. Analytical balance, accurate to 0·5 mg.

3.3. Dry containers of non-corrodible metal or of glass with lids ensuring airtight closure; working surface allowing the test sample to be spread at about 0·3 g/cm^2.

3.4. Electrically heated isothermal oven (± 1 ºC) properly ventilated and ensuring rapid temperature regulation [3].

3.5. Adjustable electrically heated vacuum oven fitted with an oil pump and either a mechanism for introducing hot dried air or a drying agent (e.g. calcium oxide).

3.6. Desiccator with a thick perforated metal or porcelain plate, containing an efficient drying agent.

4. Procedure

N.B.: The operations described in this section must be carried out immediately after opening the packages of samples. Analysis must be carried out at least in duplicate.

4.1. *Preparation*

4.1.1. *Feedingstuffs other than those coming under 4.1.2 and 4.1.3*

Take at least 50 g of the sample. If necessary, crush or divide in such a way as to avoid any variation in moisture content (see 6).

4.1.2. *Cereals and groats*

Take at least 50 g of the sample. Grind into particles of which at least 50% will pass through a 0·5 mm mesh sieve and will leave no more than 10% reject on a 1 mm round-meshed sieve.

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[1] OJ No 127, 30.9.1966, p. 3025/66.
[2] OJ No L 239, 28.9.1968, p. 2.
[3] For the drying of cereals, flour, groats and meal, the oven must have a thermal capacity such that, when pre-set at 131 ºC, it will return to that temperature in less than 45 minutes after the maximum number of test samples have been placed inside to dry simultaneously. Ventilation must be such that, when as many samples of common wheat as it can contain are dried for two hours, the results differ from those obtained after four hours of drying by less than 0·15%.

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4.1.3. *Feedingstuffs in liquid or paste form, feedingstuffs predominantly composed of oils and fats*

Take about 25 g of the sample, weigh to the nearest 10 mg, add an appropriate quantity of anhydrous sand weighed to the nearest 10 mg and mix until a homogeneous product is obtained.

4.2. *Drying*

4.2.1. *Feedingstuffs other than those coming under 4.2.2 and 4.2.3*

Weigh a container (3.3) with its lid to the nearest 0·5 mg. Weigh into the weighed container, to the nearest 1 mg, about 5 g of the sample and spread evenly. Place the container, without its lid, in the oven preheated to 103 ºC. To prevent the oven temperature from falling unduly, introduce the container as rapidly as possible. Leave to dry for four hours reckoned from the time when the oven temperature returns to 103 ºC. Replace the lid on the container, remove the latter from the oven, leave to cool for 30 to 45 minutes in the desiccator (3.6) and weigh to the nearest 1 mg.

For feedingstuffs composed predominantly of oils and fats, dry in the oven for an additional 30 minutes at 130 ºC. The difference between the two weighings must not exceed 0·1% of moisture.

4.2.2. *Cereals, flour, groats and meal*

Weigh a container (3.3) with its lid to the nearest 0·5 mg. Weigh into the weighed container, to the nearest 1 mg, about 5 g of the crushed sample and spread evenly. Place the container, without its lid, in the oven preheated to 130 ºC. To prevent the oven temperature from falling unduly, introduce the container as rapidly as possible. Leave to dry for two hours reckoned from the time when the oven temperature returns to 130 ºC. Replace the lid on the container, remove the latter from the oven, leave to cool for 30 to 45 minutes in the desiccator (3.6) and weigh to the nearest 1 mg.

4.2.3. *Compound feedingstuffs containing more than 4% of sucrose or lactose: straight feedingstuffs such as locust beans, hydrolized cereal products, malt seeds, dried beet chips, fish and sugar solubles; compound feedingstuffs containing more than 25% of mineral salts including water of crystallization.*

Weigh a container (3.3) with its lid to the nearest 0·5 mg. Weigh into the weighed container, to the nearest 1 mg, about 5 g of the sample and spread evenly. Place the container, without its lid, in the vacuum oven (3.5) preheated to between 80 ºC and 85 ºC. To prevent the oven temperature from falling unduly, introduce the container as rapidly as possible.

Bring the pressure up to 100 Torr and leave to dry for four hours at this pressure, either in a current of hot, dry air or using a drying agent (about 300 g for 20 samples). In the latter instance, disconnect the vacuum pump when the prescribed pressure has been reached. Reckon drying time from the moment when the oven temperature returns to 80 ºC to 85 ºC. Carefully bring the oven back to atmospheric pressure. Open the oven, place the lid on the container immediately, remove the container from the oven, leave to cool for 30 to 45 minutes in the desiccator (3.6) and weigh to the nearest 1 mg. Dry for an additional 30 minutes in the vacuum oven at 80 ºC to 85 ºC and reweigh. The difference between the two weighings must not exceed 0·1% of moisture.

4.3. *Preliminary drying*

4.3.1. *Feedingstuffs other than those coming under 4.3.2*

Solid feedingstuffs with a high moisture content which makes crushing difficult must be subjected to preliminary drying as follows:

Weigh 50 g of *uncrushed* sample to the nearest 10 mg (compressed or agglomerated feedingstuffs may be roughly divided if necessary) in a suitable container (e.g. a 20 × 12 cm aluminium plate with a 0·5 cm rim). Leave to dry in an oven from 60 ºC to 70 ºC until the moisture content has been reduced to between8% and 12%. Remove from the oven, leave to cool uncovered in the laboratory for one hour and weigh to the nearest

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10 mg. Crush immediately as indicated in 4.1.1 and dry as indicated in 4.2.1 or 4.2.3 according to the nature of the feedingstuff.

4.3.2. *Cereals*

Grain with a moisture content of over 17% must be subjected to preliminary drying as follows:

Weigh 50 g of *unground* grain to the nearest 10 mg in a suitable container (e.g. a 20 × 12 cm aluminium plate with a 0·5 cm rim). Leave to dry for 5 to 7 minutes in an oven at 130 ºC. Remove from the oven, leave to cool uncovered in the laboratory for two hours and weigh to the nearest 10 mg. Grind immediately as indicated in 4.1.2 and dry as indicated in 4.2.2.

5. **Calculation of results**

The moisture content, as a percentage of the sample, is calculated by using the following formulae:

5.1. *Drying without preliminary drying*

$$(E - m) \cdot \frac{100}{E}$$

where:

E = initial mass, in grammes, of the test sample,

m = mass, in grammes, of the dry test sample.

5.2. *Drying with preliminary drying*

$$\left[\frac{(M' - m)\ M}{M'} + E - M\right] \cdot \frac{100}{E} = 100\left(1 - \frac{Mm}{EM'}\right)$$

where:

E = initial mass, in grammes, of the test sample,

M = mass, in grammes, of the test sample after preliminary drying,

M′ = mass, in grammes, of the test sample after crushing or grinding,

m = mass, in grammes, of the dry test sample.

5.3. *Repeatibility*

The difference between the results of two parallel determinations carried out on the same sample should not exceed 0·2% of moisture.

6. **Observation**

If crushing proves necessary and if this is seen to alter the moisture content of the product, the results of the analysis of the components of the feedingstuff must be corrected on the basis of the moisture content of the sample in its initial state.

II. DETERMINATION OF VOLATILE NITROGENOUS BASES

A. MICRODIFFUSION

1. **Purpose and Scope**

This method makes it possible to determine the content of volatile nitrogenous bases, expressed as ammonia, in feedingstuffs.

2. **Principle**

The sample is extracted with water and the solution clarified and filtered. The volatile nitrogenous bases are displaced by microdiffusion using a solution of potassium carbonate, collected in a solution of boric acid and titrated with sulphuric acid.

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3. **Reagents**

3.1. 20% (w/v) solution of trichloroacetic acid.

3.2. Indicator: dissolve 33 mg of bromocresol green and 65 mg of methyl red in 100 ml of 95%—96% (v/v) of ethanol.

3.3. Boric acid solution: in a 1 litre graduated flask dissolve 10 g of boric acid A.R. in 200 ml of 95%—96% (v/v) ethanol and 700 ml of water. Add 10 ml of indicator (3.2.). Mix and, if necessary, adjust the colour of the solution to light red by adding a solution of sodium hydroxide. 1 ml of this solution will fix a maximum of 300 μg of NH_3.

3.4. Saturated potassium carbonate solution: dissolve 10 Og of potassium carbonate A.R. in 100 ml of boiling water. Leave to cool, filter.

3.5. Sulphuric acid 0·02 N.

4. **Apparatus**

4.1. Mixer (tumbler): approximately 35 to 40 r.p.m.

4.2. Glass or plastic Conway cells (see diagram).

4.3. Microburettes graduated in 1/100 ml.

5. **Procedure**

Weigh 10 g of sample to the nearest 1 mg and place with 100 ml of water in a 200 ml graduated flask. Mix in the tumbler for 30 minutes. Add 50 ml of trichloroacetic acid solution (3.1), make up to volume with water, shake vigorously and filter through a pleated filter.

Using a pipette, introduce 1 ml of boric acid solution (3.3) into the central part of the Conway cell and 1 ml of the sample filtrate into the crown of the cell. Cover partially with the greased lid. Drop 1 ml of saturated potassium carbonate solution (3.4) quickly into the crown and close the lid so that the cell is airtight. Turn the cell carefully rotating it in a horizontal plane so that the two reagents are mixed. Leave to incubate either for at least four hours at room temperature or for one hour at 40 ºC.

Using a microburette (4.3), titrate the volatile bases in the boric acid solution with sulphuric acid 0·02 N (3.5).

Carry out a blank test using the same procedure but without a sample to be analysed.

6. **Calculation of results**

1 ml of H_2SO_4 0·02 N corresponds to 0·34 mg of ammonia.

Express the result as a precentage of the sample.

Repeatibility

The difference between the results of two parallel determinations carried out on the same sample should not exceed:

10%, in relative value, for ammonia contents of less than 1·0%;

0·1%, in absolute value, for ammonia contents of 1·0% or more.

7. **Observation**

If the ammonia content of the sample exceeds 0·6%, dilute the initial filtrate.

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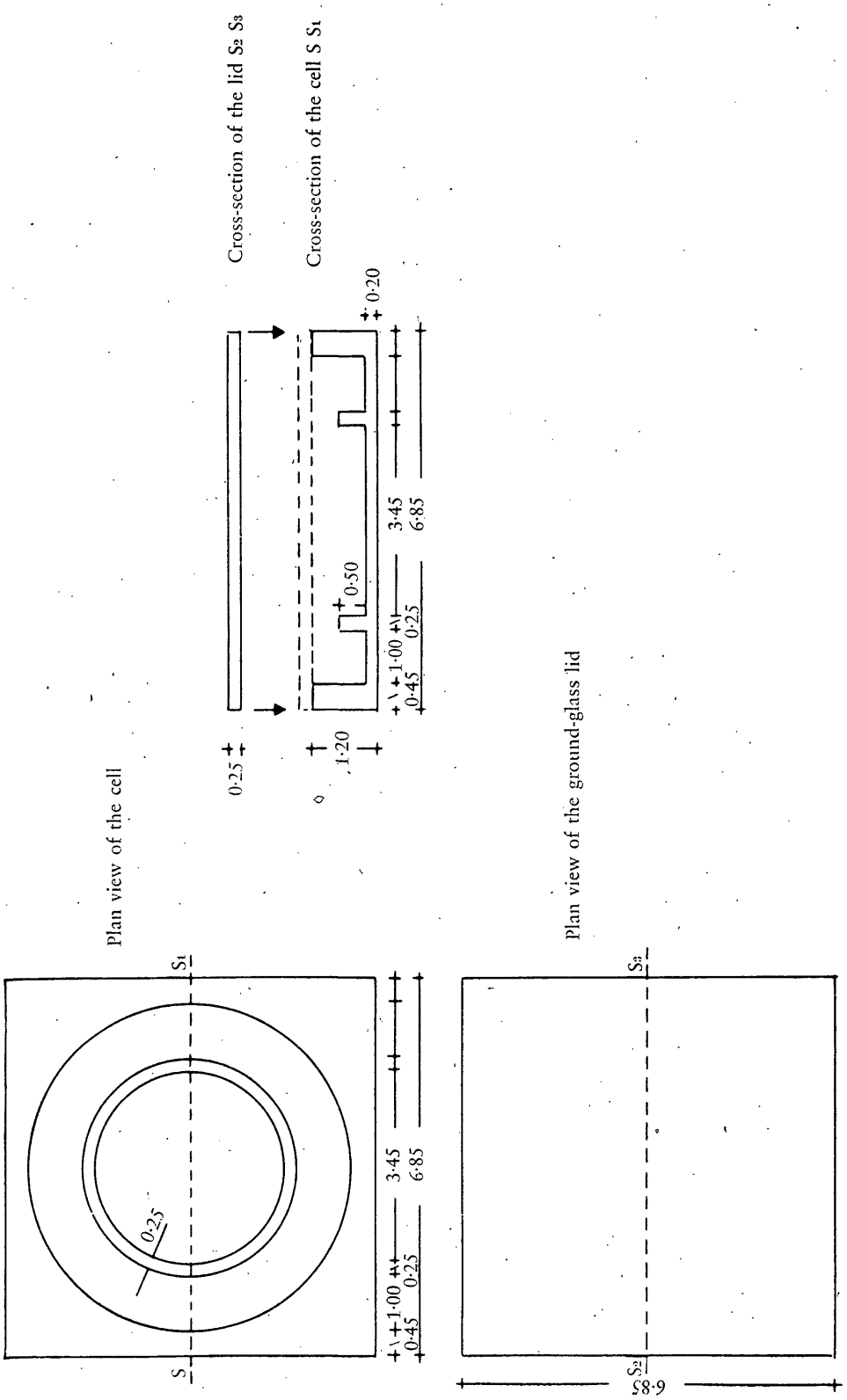
CONWAY CELL

Scale 1/1



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B. BY DISTILLATION

1. **Purpose and Scope**

This method makes it possible to determine the content of volatile nitrogenous bases, expressed as ammonia, in fish-meal containing practically no urea. It is applicable only to ammonia contents of less than 0·25%.

2. **Principle**

The sample is extracted with water and the solution clarified and filtered. The volatile nitrogenous bases are displaced at boiling point by adding magnesium oxide and collected in a specific quantity of sulphuric acid, the excess of which is back-titrated with a solution of sodium hydroxide.

3. **Reagents**

3.1. 20% (w/v) solution of trichloroacetic acid.

3.2. Magnesium oxide A.R.

3.3. Anti-foaming emulsion (e.g. silicone).

3.4. Sulphuric acid 0·1 N.

3.5. Sodium hydroxide solution 0·1 N.

3.6. 0·3% (w/v) solution of methyl red in 95%—96% (v/v) ethanol.

4. **Apparatus**

4.1. Mixer (tumbler): approximately 35 to 40 r.p.m.

4.2. Distilling apparatus of the Kjeldahl type.

5. **Procedure**

Weigh 10 g of the sample to the nearest 1 mg and place with 100 ml of water in a 200 ml graduated flask. Mix in the tumbler for 30 minutes. Add 50 ml of trichloroacetic acid solution (3.1), make up to volume with water, shake vigorously and filter through a pleated filter.

Take a quantity of clear filtrate appropriate for the presumed content of volatile nitrogenous bases (100 ml is usually suitable). Dilute to 200 ml and add 2 g of magnesium oxide (3.2) and a few drops of anti-foaming emulsion (3.3).The solution should be alkaline to litmus paper; otherwise add some magnesium oxide (3.2). Distil about 150 ml of the solution in the Kjeldahl apparatus and collect the distillate in an Erlenmeyer flask containing an accurately measured volume (25 to 50 ml) of sulphuric acid 0·1 N (3.4). While distilling, avoid overheating of the sides. Boil the sulphuric acid solution for two minutes, cool it and back-titrate the excess sulphuric acid with the sodium hydroxide solution 0·1 N (3.5) in the presence of the methyl red indicator (3.6).

Carry out a *blank test* using the same procedure but without a sample to be analysed.

6. **Calculation of results**

1 ml of H_2SO_4 0·1 N corresponds to 1·7 mg of ammonia.

Express the result as a percentage of the sample.

Repeatibility

The difference between the results of two parallel determinations carried out on the same sample should not exceed, in relative value, 10% of ammonia.

III. **DETERMINATION OF TOTAL PHOSPHORUS**

PHOTOMETRIC METHOD

1. **Purpose and Scope**

This method makes it possible to determine the content of total phosphorus in feedingstuffs. It is particularly appropriate for the analysis of products low in phosphorus. In certain cases (product rich in phosphorus), a gravimetric method may be used.

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2. **Principle**

The sample is mineralised, either by dry combustion (in the case of organic feedingstuffs) or by acid digestion (in the case of mineral compounds and liquid feedingstuffs), and placed in an acid solution. The solution is treated with molybdovanadate reagent. The optical density of the yellow solution thus formed is measured in a spectrophotometer at 430 nm.

3. **Reagents**

3.1. Calcium carbonate A.R.

3.2. Hydrochloric acid A.R., d: 1·1 (approx 6 N).

3.3. Nitric acid A.R., d: 1·045.

3.4. Nitric acid A.R., d: 1·38 to 1·42.

3.5. Sulphuric acid A.R., d: 1·84.

3.6. Molybdovanadate reagent: mix 200 ml of ammonium heptamolybdate solution (3.6.1), 200 ml of ammonium monovanadate solution (3.6.2) and 134 ml of nitric acid (3.4) in a 1 litre graduated flask. Make up to volume with water.

3.6.1. Ammonium heptamolybdate solution: dissolve in hot water 100 g of ammonium heptamolybdate A.R.

$(NH_4)_6Mo_7O_{24}.^4H_2O$. Add 10 ml of ammonia (d: 0·91) and make up to 1 litre with water.

3.6.2. Ammonium monovanadate solution: 2·35 g of ammonium monovanadate A.R. NH_4VO_3 dissolve in 400 ml of hot water. Stirring constantly, slowly add 20 ml of dilute nitric acid (7 ml of HNO_3 (3.4) + 13 ml of H_2O and make up to 1 litre with water.

3.7. Standard solution of 1 mg phosphorus per ml: dissolve 4·387 g of potassium dihydrogen phosphate A.R. KG_2PO_4 in water. Make up to 1 litre with water.

4. **Apparatus**

4.1. Silica or porcelain ashing crucibles.

4.2. Electric muffle-furnace with thermostat set at 550 ºC.

4.3. 250 ml Kjeldahl flask.

4.4. Graduated flasks and precision pipettes.

4.5. Spectrophotometer.

4.6. Test tubes about 16 mm in diameter, with stoppers graded to a diameter of 14·5 mm; capacity: 25 to 30 ml.

5. **Procedure**

5.1. *Preparation of the solution*

According to the nature of the sample, prepare a solution as indicated in 5.1.1 or 5.1.2.

5.1.1. *Usual procedure*

Weigh 1 g or more of the sample to the nearest 1 mg. Place the test sample in a Kjeldahl flask, add 20 ml of sulphuric acid (3.5), shake to impregnate the substancecompletely with acid and to prevent it from sticking to the sides of the flask, heat and keep at boiling point for 10 minutes. Leave to cool slightly, add 2 ml of nitric acid (3.4), heat gently, leave to cool slightly, add a little more nitric acid (3.4) and bring back to boiling point. Repeat this procedure until a colourless solution is obtained. Cool, add a little water, decant the liquid into a 500 ml graduated flask, rinsing the Kjeldahl flask with hot water. Leave to cool, make up to volume with water, homogenise and filter.

5.1.2. *Samples containing organic substances and free from calcium and magnesium dihydrogen phosphates*

Weigh about 2·5 g of the sample to the nearest 1 mg in an ashing crucible. Mix the test sample until completely merged with 1 g of calcium carbonate (3.1). Ash in the oven at 550 ºC ± 5 ºC until white or grey ash is obtained (a little charcoal does not matter).

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Transfer the ash into a 250 ml beaker. Add 20 ml of water and hydrochloric acid (3.2) until effervescence ceases. Add a further 10 ml of hydrochloric acid (3.2). Place the beaker on a sand bath and evaporate until dry to make the silica insoluble. Redissolve the residue in 10 ml of nitric acid (3.3) and boil on the sand bath for 5 minutes without evaporating until dry. Decant the liquid into a 500 ml graduated flask, rinsing the beaker several times with hot water. Leave to cool, make up to volume with water, homogenise and filter.

5.2. *Development of coloration and measurement of optical density*

Dilute an aliquot part of the filtrate obtained by 5.1.1 or 5.1.2 to obtain a phosphorus concentration of not more than 40 µg/ml. Place 10 ml of this solution in a test tube (4.6) and add 10 ml of molybdovanadate reagent (3.6). Homogenise and leave to stand for at least 10 minutes at 20 ºC. Measure the optical density in a spectrophotometer at 430 nm against a solution obtained by adding 10 ml of the molybdovanadate reagent (3.6) to 10 ml of water.

5.3. *Calibration curve*

From the standard solution (3.7) prepare solutions containing respectively 5, 10, 20, 30 and 40 µg of phosphorus per ml. Take 10 ml of each of these solutions and add thereto 10 ml of molybdovanadate reagent (3.6). Homogenise and leave to stand for at least 10 minutes at 20 ºC. Measure the optical density as indicated in 5.2.

Trace the calibration curve by plotting the optical densities against the corresponding quantities of phosphorus. For concentrations between 0 and 40 µg/ml, the curve will be linear.

6. **Calculation of results**

Determine the amount of phosphorus in the test sample by using the calibration curve.

Express the result as a percentage of the sample.

Repeatibility

The difference between the results of two parallel determinations carried out on the same sample should not exceed:

3%, in relative value, for phosphorus contents of less than 5%;

0·15%, in absolute value, for phosphorus contents of 5% or more.

IV. DETERMINATION OF CRUDE OILS AND FATS

1. **Purpose and Scope**

This method makes it possible to determine the content of crude oils and fats in feedingstuffs. It does not cover the analysis of the oil seeds and oleaginous fruit defined in Council Regulation 136/66/EEC of 22 September 1966. The determination of the oil content of those products is described in Annex V to Commission Regulation (EEC) No 1470/68 of 23 September 1968.

Either one of two methods may be used, depending on the nature of the feedingstuff.

1.1. *Method A* (extraction by ether): applicable to all feedingstuffs other than those coming under 1.2

1.2. *Method B:* applicable to feedingstuffs from which oils and fats cannot be totally extracted with diethyl either without prior hydrolysis, to feedingstuffs of animal origin, glutens, dried potato pulps, dried brewing and distilling dreys and waste, dried yeasts, waste from biscuits, bread and cooked foods, milk products and feedingstuffs containing a high proportion of such products (at least 40%) and to compound feedingstuffs enriched with fats.

2. **Principle**

2.1. *Method A:* The oils and fats are extracted with diethyl ether. The solvent is distilled off and the residue dried and weighed.

2.2. *Method B:* The sample is hydrolised when hot with hydrochloric acid. The solution is cooled and filtered. The residue is washed and dried and extracted with diethyl ether using Method A.

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3. **Reagents**

3.1. Anhydrous diethyl ether, d: 0·720, B.P.: 34·5 ºC, virtually free from peroxides

3.2. Anhydrous sodium sulphate A.R.

3.3. Hydrochloric acid 3N.

3.4. Filtration aid, e.g. Kieselgur, Hyflo-supercel.

3.5. Carbon tetrachloride A.R.

4. **Apparatus**

4.1. Soxhlet-type extractor or equivalent apparatus.

4.2. Explosion-proof heating apparatus with temperature control.

4.3. Vacuum drying oven (less than 100 Torr).

5. **Procedure**

5.1. *Method A:* (see 7.1)

Weigh 5 g of the sample to the nearest 1 mg and mix with 2 g to 3 g (or more, if necessary) of anhydrous sodium sulphate (3.2). Place the mixture in an extraction thimble free from oils and fats and cover with a fat-free wad of cottonwool. (The mixing may be carried out in the thimble.)

Place the thimble in an extracter (4.1) and extract for six hours with diethyl ether (3.1). If a Soxhlet-type extractor is used, regulate the heating to obtain at least 15 siphonings per hour. Collect the ether extract in a dry, weighed flask containing fragments of pumice stone [1].

Distil off the ether and dry the evaporation residue for one and a half hours in the vacuum drying oven (4.3) at 75 ºC. Cool in a desiccator and weigh. Dry again for 30 minutes to ensure that the weight of the oil and fat remains constant (loss in weight must be less than 1 mg).

5.2. *Method B*

Weigh 2·5 g of the sample to the nearest 1 mg (see 7.2) and place in a 400 ml beaker or a 300 ml Erlenmeyer flask. Add 100 ml of hydrochloric acid 3 N (3.3) and fragments of pumice stone. Cover the beaker with a watch glass or fit the Erlenmeyer flask with a reflux condenser. Bring the mixture to a gentle boil over a low flame or a hot plate and keep it there for one hour. Do not allow the product to stick to the sides of the container.

Cool and add a quantity of filtration aid (3.4) sufficient to prevent any loss of oil and fat during filtration. Filter through a moistened, fat-free, double filter paper. Wash the residue in cold water until the acid reaction has ceased. Check that the filtrate does not contain any oil or fats. Their presence in the filtrate indicates that the sample must be extracted with diethyl ether, using the method given in 5.1, before hydrolysis.

Place the double filter paper containing the residue on a watch glass and dry for one and a half hours in the oven at 95 ºC to 98 ºC.

Place the double filter paper and the dry residue in an extraction thimble, extract with diethyl ether and proceed as indicated in the second paragraph of 5.1.

6. **Calculation of results**

Express the result as a percentage of the sample.

Repeatibility

The difference between the results of two parallel determinations carried out on the same sample should not exceed 0·3% of oil or fat.

[1] Where the oil or fat has to undergo subsequent quality tests, replace the fragments of pumice stone by glass beads.

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7. **Observations**

7.1. For products with a high content of oils and fats and which are difficult to crush or unsuitable for drawing a homogeneous reduced test sample, proceed as follows. Weigh 20 g of the sample to the nearest 1 mg and mix with 10 g or more of anhydrous sodium sulphate (3.2). Extract with diethyl ether (3.1) as indicated in 5.1. Make up the extract obtained to 500 ml with carbon tetrachloride (3.5) and homogenise. Take 50 ml of the solution and place in a small, dry, weighed flask containing fragments of pumice stone [1]. Distil off the solvent, dry and proceed as indicated in the last paragraph of 5.1. Eliminate the solvent from the extraction residue left in the thimble and crush the residue to a fineness of 1 mm. Return the product to the extraction thimble (do not add sodium sulphate), extract with diethyl ether and proceed as indicated in the second and third paragraphs of 5.1.

Calculate the result as a percentage of the sample, taking into account the aliquot part used for the first extraction, by using the following formula:

$$(10\ a + b) \cdot 5$$

where:

a = ether extract, in grammes, of the aliquot part after the first extraction;

b = ether extract, in grammes, after the second extraction.

7.2. For products low in oils and fats the test sample may be increased to 5 g.

[1] Where the oil or fat has to undergo subsequent quality tests, replace the fragments of pumice stone by glass beads.