EUROPEAN COMMUNITIES (MARKETING OF FEEDINGSTUFFS) REGULATIONS 1984

I, AUSTIN DEASY, Minister for Agriculture, in exercise of the powers conferred on me by section 3 of the European Communities Act, 1972 (No. 27 of 1972), and for the purpose of giving effect to Council Directive No. 77/101/EEC of 23 November, 1976 1 as amended in the manner specified in relation thereto in Regulation 2 of the following regulations, and Council Directive No. 79/373/EEC of 2 April, 1979 2 as amended in the manner specified in relation thereto in the said Regulation 2, hereby make the following regulations:
1 O.J. No. L32/1, 3 February 1977.
2 O.J. No. L86/30, 6 April 1979.

REG 1

1. (1) These Regulations may be cited as the European Communities (Marketing of Feedingstuffs) Regulations, 1984.
(2) Those Regulations, other than Regulation 8 (1) (b) (vi) in so far as it requires to be made declarations of either lysine in compound feedingstuffs for swine or methionine and cystine in compound feedingstuffs for poultry, shall come into effect on the 1st day of August, 1985.
(3) Regulation 8 (1) (b) (vi) of these Regulations shall, in so far as it requires to be made the declarations referred to in paragraph (2) of this Regulation, come into effect on the 1st day of August, 1986.

REG 2

2. (1) In these Regulations—
"authorised person" means a person appointed in writing by the Minister to be an authorised person for the purposes of these Regulations;
"the Directive of 1976" means Council Directive No. 77/101/EEC of 23 November, 1976 1, as amended by Council Directive No. 79/372/EEC of 2 April, 19793, First Commission Directive No. 79/797/EEC of 10 August, 1979 4, Second Commission Directive No. 80/510/EEC of 2 May, 1980 5, Third Commission Directive No. 82/937/EEC of 21 December, 1982 6, and Commission Directive No. 83/87/EEC of 21 February, 1983 7;
1 O.J. No. L32/1, 3 February 1977.
3 O.J. No. L86/29, 6 April 1979.
4 O.J. No. L239/53, 22 September 1979.
5 O.J. No. L126/12, 21 May 1980.
6 O.J. No. L383/11, 31 December 1982.
7 O.J. No. L56/31, 3 March 1983.
"the Directive of 1979" means Council Directive No. 79/373/EEC of 2 April, 1979 2, as amended by First Commission Directive No. 80/509/EEC of 2 May, 1980 8 Commission Directive No. 80/511/EEC of 2 May, 1980 9, Second Commission Directive No. 80/695/EEC of 27 June, 1980 10, and Third Commission Directive No. 82/957/EEC of 22

December, 1982 11.
2 O.J. No. L86/30, 6 April 1979.
8 O.J. No. L126/9, 21 May 1980.
9 O.J. No. L126/14, 21 May 1980.
10 O.J. No. L188/23, 22 July 1980.
11 O.J. L386/42, 31 December 1982.
"the Minister" means the Minister for Agriculture;
"straight feedingstuffs" means the various vegetable and animal products in their natural state, fresh or preserved, and their derivatives after industrial processing, as well as the various organic and inorganic substances, intended for oral animal feeding;
"State Chemist" means the head of the State Laboratory or a person authorised by him in writing to perform the functions assigned to the State Chemist by these Regulations.
(2) A word or expression, other than "straight feedingstuff", that is used in these Regulations and is also used in the Directive of 1976 or the Directive of 1979 has the meaning in these Regulations that it has in the Directive of 1976 or the Directive of 1979, as may be appropriate.
(3) The Fertilisers, Feedingstuffs and Mineral Mixtures Regulations, 1957 (S.I. No. 264 of 1957), other than Regulation 13 thereof, shall, in so far as they apply to feedingstuffs, straight feedingstuffs or compound feedingstuffs to which these Regulations apply, cease to have effect.
(4) Subsequent references in these Regulations to feedingstuffs, straight feedingstuffs or compound feedingstuffs shall, unless the context otherwise requires, each be construed as references to feedingstuffs to which these Regulations apply, or, in the case of references to straight feedingstuffs, references to straight feedingstuffs which are feedingstuffs to which these Regulations apply, or, in the case of references to compound feedingstuffs, references to compound feedingstuffs which are feedingstuffs to which these Regulations apply.

REG 3

3. These Regulations shall apply to any feedingstuff other than—
(a) a straight feedingstuff which is marked in such a way as to establish that it is intended for export,
(b) any compound feedingstuff in respect of which—
(i) it is proved at least by some appropriate marking that it is intended for export,
or
(ii) it is proved in the manner specified in Article 14 (c) of the Directive of 1979 that it is intended for animals kept for scientific or experimental purposes,
(c) wheat, barley, oats, hay, silage, roof crops or any other primary agricultural produce which is sold by the producer for his own benefit and on his own account.

REG 4

4. Feedingstuffs (whether straight or compound) may be marketed only if they are wholesome, unadulterated and of merchantable quality, and they shall not represent a danger to animal or human health and shall neither be represented nor marketed in a manner liable to mislead.

REG 5

5. A person shall not market a straight feedingstuff unless—
(a) the requirements of Regulation 4 of these Regulations are complied with in relation to it,
(b) it complies with each of the general provisions laid down in Part A of the Annex to the Directive of 1976, other than paragraphs 1.2, 2.5 and 2.6, and paragraph 1.3 in so far as it applies to feedingstuffs listed in items 3.2.8 of Part B of the said Annex,
(c) where appropriate, the requirements of Article 11 of the Directive of 1976 are complied with in relation to it,
(d) in case the feedingstuff is not a product or substance referred to in Article 14 (a) of the Directive of 1976, the content of ash insoluble in hydrochloric acid (HCl) does not exceed the maximum specified in column (6) of Part I of the First Schedule to these Regulations opposite the mention of the feedingstuff in column (2) of that Part,
(e) in case the feedingstuff is a product or substance referred to in the said Article 14 (a) whose content of ash insoluble in hydrochloric acid (HCl) exceeds that specified in the said column (6) opposite the mention in the said column (2) of the feedingstuff and it is marketed to a person who for the time being holds a licence granted under the Fertilisers, Feeding Stuffs and Mineral Mixtures Regulations, 1957 (S.I. No. 264 of 1957), the content of such ash is declared on the package or container or on a label attached thereto or on an accompanying document, and
(f) in case the feedingstuff is a feedingstuff specified in column (2) of Part I of the First Schedule to these Regulations opposite a reference number in column (1) of that Part—
(i) it is marketed under, and only under, the word or words used as its name in the said column (2), and
(ii) if it has undergone a process which is not indicated by name, the requirements of paragraph 1.1 of Part A of the Annex to the Directive of 1976 are complied with in relation to it, and
(iii) it corresponds to the description set out in column (3) of the said Part I opposite that reference number.

REG 6

6. (1) A person shall not market a compound feedingstuff unless—
(a) it is in a sealed package or container, and
(b) such package or container is sealed in such a way that, when the package or container is opened, the seal is damaged and cannot be reused, and

(c) each of the requirements specified in paragraph (2) of this Regulation, or where appropriate, each of such requirements as apply to it, is complied with.
(2) The following are the requirements referred to in paragraph (1) of this Regulation—
(a) the requirements of Regulation 4 of these regulations shall be complied with in relation to the feedingstuff concerned,
(b) such feedingstuff shall comply with the provisions laid down in points 1 and 2 of the Annex to the Directive of 1979,
(c) such feedingstuff shall not contain, as an ingredient, any substance specified in Part II of the First Schedule to these Regulations,
(d) where appropriate, the requirements of Article 11 of the Directive of 1979 are complied with in relation to it,
(e) the level in such feedingstuff of ash insoluble in hydrochloric acid (HCl) shall not exceed 3.3% of the dry matter in case the feedingstuff is composed mainly of rice by-products, or 2.2% of the dry matter in case the feedingstuff is not so composed:
Provided that the aforesaid levels may be exceeded in, but only in, the following circumstances:
(i) if the relevant feedingstuff is a compound feedingstuff containing mineral binding agents authorised under the European Communities (Feeding Stuffs) (Additives) Regulations, 1974 to 1983, or
(ii) if such feedingstuff is a mineral compound feedingstuff, or
(iii) if such feedingstuff is a compound feedingstuff containing more than 50% of sugar beet chips or pulp, and
(iv) where the level in any such feedingstuff of ash insoluble in hydrochloric acid (HCl) exceeds 3.3% of the dry matter, such level is declared as a percentage of the feedingstuff as such on the relevant package, container or label or on an accompanying document.

REG 7

7. (1) Notwithstanding Regulation 6 of these Regulations but subject to Regulation 8 (2) (b) of these Regulations, compound feedingstuffs may be marketed in bulk or in unsealed packages or containers in the case of—
(a) deliveries between producers of compound feedingstuffs,
(b) deliveries from producers of compound feedingstuffs to packaging firms,
(c) compound feedingstuffs obtained by mixing grain or whole fruit,
(d) blocks or licks, or
(e) quantities of compound feedingstuffs not exceeding 50 kilograms in weight which are intended for the final user and are taken directly from a package or container which before being opened complied with the said Regulation 6.
(2) Notwithstanding Regulation 6 of these Regulations, but subject to

Regulation 8 (2) (b) of these Regulations, compound feedingstuffs may be marketed in bulk or in unsealed containers, but not in unsealed packages, in the case of—
(a) compound feedingstuffs delivered directly from the producer to the final user,
(b) molassed feedingstuffs consisting of not more than three ingredients, or
(c) pelleted compound feedingstuffs.

REG 8

8. (1) Subject to paragraphs (2) and (3) of this Regulation, feedingstuffs shall be marketed only if the following particulars, which shall be clearly visible, legible and indelible, are set out on the package or container or on a label attached thereto:
(a) in case a feeding stuff is a straight feedingstuff—
(i) unless the feedingstuff is marketed to a person described in Regulation 5 (e) of these Regulations, the words "straight feedingstuff",
(ii) the particulars specified in column (4) of Part I of the First Schedule to these Regulations opposite where the name of the feedingstuff appears in column (2) thereof together with the words or word, as may be appropriate, used as its name in the said column (2),
(iii) the particulars specified in paragraphs (e) and (f) of Article 7 (1) of the Directive of 1976,
(iv) where appropriate, the particulars specified in paragraphs 1.1 and 2 of Part A of the Annex to the Directive of 1976 together with, in case the feedingstuff is a feedingstuff listed in item 2.9.2 of Part B of the said Annex, the particulars specified in paragraph 1.3 of the said Part A,
(v) in case the feedingstuff is a feedingstuff described in paragraph (e) of Regulation 5 of these Regulations, the declaration required by the said Regulation 5.
(b) in case the feedingstuff is a compound feedingstuff—
(i) where the feedingstuff is a complete feedingstuff, the description "complete feedingstuff",
(ii) where the feedingstuff is a complementary feedingstuff—
(I) if it is a complementary mineral feedingstuff, the description "complementary mineral feedingstuff"
(II) if it is a complementary molassed feedingstuff, the description "complementary molassed feedingstuff"
and
(III) if it is neither a complementary mineral feedingstuff nor a complementary molassed feedingstuff, the description "complementary feedingstuff",
(iii) the particulars listed in paragraphs (b), (c), (d), (f) and (g) of Article 5 (1) of the Directive of 1979,
(iv) except in the case of feedingstuffs which are intended for pet animals, the date of manufacture,
(v) in case the feedingstuff is a milk replacer (suckling feed), the milk powder content,
(vi) the declarations listed in column (2) of part III of the First Schedule to these Regulations opposite the mention in column

(1) of that Part of the relevant type of feedingstuff, and
(vii) the declaration required by Regulation 6 (1) of these Regulations and referred to in Regulation 6 (2) (e) (iv) of these Regulations:
Provided that:
(I) in case of small quantities of compound feedingstuffs intended for the final user, in lieu of complying with the requirements of subparagraph (b) of this paragraph, the particulars specified in that subparagraph may be brought to the purchasers' attention by means of an appropriate notice; and
(II) in case the feedingstuff is constituted from no more than three ingredients, the particulars referred to in subparagraph (b) and, where appropriate, either or both of the following, namely, subparagraphs (c) or (d) of the said Article 5 (1) shall not be required if the ingredients used in the feedingstuff appear clearly in a description of the feedingstuff contained in the aforesaid particulars.
(2) (a) In relation to straight feedingstuffs marketed in bulk, paragraph (1) of this Regulation shall be construed as requiring the particulars specified in that paragraph to be set out in an accompanying document.
(b) In relation to compound feedingstuffs duly marketed in tankers or similar vehicles or otherwise in bulk, paragraph (1) of this Regulation shall be construed as requiring the particulars to be set out in an accompanying document.
(3) The declarations referred to in paragraph (1) (b) of this Regulation shall each comply with the requirements of point 1 of the Annex to the Directive of 1979.

REG 9

9. (1) A person who markets a straight feedingstuff shall not put on the package, container or label thereof or on an accompanying document, in conjunction with any of the particulars set out in compliance with the requirements of Regulation 8 (1) of these Regulations, additional particulars other than the following, namely—
(a) the particulars listed in Article 7 (5) of the Directive of 1976 (other than paragraph (g)),
and
(b) the declarations listed in column (5) of Part I of the First Schedule to these Regulations opposite the mention in column (2) thereof of the relevant feedingstuff.
(2) A person who markets a compound feedingstuff shall not put on the package, container, label or accompanying document thereof in conjunction with the particulars set out in compliance with the requirements of Regulation 8 (1) (b) of these Regulations additional particulars other than—
(a) the particulars listed in Article 5 (5) of the Directive of 1979,
(b) the trade name of the product,

(c) each of the ingredients in descending order of its proportion by weight,
(d) the declarations listed in column (3) of Part III of the First Schedule to these Regulations opposite the mention in column (1) thereof of the relevant type of feedingstuff,
(e) where it is appropriate, an indication that the compound feedingstuff conforms to a standard or standards regarding analytical characteristics which for the time being stands or stand recommended by the Minister in relation to a particular category or particular categories of compound feedingstuffs.
(3) Paragraph (3) of Regulation 8 of these Regulations shall apply to a declaration, other than a declaration referred to in paragraph (1) (b) of that Regulation, which pursuant to these Regulations is put on a package, container, label or accompanying document as it applies to a declaration so referred to.
10. (a) Where information referred to in Article 7 (7) of the Directive of 1976 appears on any packaging, container, label or accompanying document to which the said Article 7 (7) relates, the information shall be given in accordance with the requirements of that Article.
(b) Where information referred to in Article 5 (8) of the Directive of 1979 appears on any packaging, container, label or accompanying document to which the said Article 5 (8) relates, the information shall be shown in accordance with the requirements of that Article.

REG 11

11. (1) Where a person has on his premises any feedingstuff (whether straight or compound) which he has purchased and which he proposes to use in the course of his farming operations, he may apply to the Minister to have a sample thereof taken for analysis.
(2) An application under this Regulation shall be—
(a) made within the period of thirty days beginning on the date on which the feedingstuff to which the application relates was delivered to the applicant, and
(b) accompanied by a fee which shall be calculated by reference to the rates specified in the Second Schedule to these Regulations.
(3) Where an application is made under this Regulation, an authorised person shall, subject to paragraph (4) of this Regulation—
(a) take and deal with a sample of the relevant feedingstuff according to the methods described in the Annex to Commission Directive No. 76/371/EEC of 1 March, 19761,
1 O.J. No. L102/1, 15 April 1976.
and
(b) give or cause to be given, or sent by registered post or by such other method as for the time being stands approved of for the purposes of this paragraph by the Minister, to the State Chemist and to the person whose name or trade name has been given pursuant to paragraph (f) of Article 7 (1) of the Directive of 1976, or, as may be appropriate, paragraph (f) of Article 5 (1) of the Directive of 1979, one of the final samples prepared pursuant to the requirements of subparagraph (a) of this paragraph.

(4) Where an application is made under this Regulation, an authorised person may, if he thinks fit, decline to take a sample if—
(a) he is not satisfied that the applicant has purchased the feedingstuff to which the application relates,
or
(b) he is not satisfied that the applicant proposes to use such feedingstuff in the course of his farming operations,
or
(c) he is not satisfied that such feedingstuff as presented for sampling is fairly representative of the feedingstuff as delivered to the applicant,
or
(d) the applicant does not furnish such information relating to such feedingstuff as the authorised person may reasonably require.
(5) Where the State Chemist receives a sample taken in pursuance of an application under this Regulation, he shall in making an analysis thereof comply with any of the requirements set out in Part I of the Third Schedule to these Regulations, as apply in the particular case and send to the applicant and to the person (other than the State Chemist) referred to in paragraph (3) (b) of this Regulation a certificate, in the form set out in Part II of the said Third Schedule, of the result of the analysis.
(6) Subject to paragraph (8) of this Regulation, all fees under this Regulation shall be paid into or disposed of for the benefit of the Exchequer in accordance with the directions of the Minister for Finance and, accordingly, the Public Offices Fees Act, 1879, shall not apply in respect thereof.
(7) Nothing in this Regulation shall be construed as requiring the State Chemist to make a test, examination or analysis regarding the presence in or absence from a sample given or sent to him pursuant to these Regulations of any particular substance, product or other thing, if in his opinion there is not in relation to such presence or absence a method of testing, examination or analysis which is sufficiently reliable or if there is not available to the State Chemist the apparatus or other means by which such a test, examination or analysis could be made.
(8) In any case in which he considers it proper so to do (not being a case in which the applicant has received a certificate under this Regulation), the Minister may refund a fee paid in relation to an application under this Regulation.
(9) For the purpose of this Regulation a feedingstuff shall not be regarded as having been delivered to a purchaser until it arrives at the destination to which it is consigned whether the consignment is by direction of the supplier or the purchaser.

REG 12

12. Every person who carries on, or is employed in connection with, the business of manufacturing, importing or marketing feedingstuffs shall—
(a) keep records of his transactions in such feedingstuffs;
(b) produce at the request of an authorised person any books, documents or records relating to such business which are in the

possession or under the control of such person;
(c) permit an authorised person to inspect and take extracts from such books, documents or records and give to the person any information which he may reasonably require in relation to any entries therein;
(d) afford to an authorised person reasonable facilities for inspecting the stock of any feedingstuff which is for the time being on any premises on which such person carries on such a business;
(e) give to an authorised person any information he may reasonably require in relation to such transactions including in particular information which he may reasonably require regarding any feedingstuff which is specified by him.

REG 13

13. Where an authorised person is satisfied that a feedingstuff which is placed on the market or which he believes will be placed on the market, does not comply with any one or more of the requirements of these Regulations, he may require either or both of the following persons, namely, the person who appears to him to have for the time being possession or control of the feedingstuff and the person whose name or trade name has been given pursuant to paragraph (f) of Article 7 (1) of the Directive of 1976, or, as may be appropriate, paragraph (f) of Article 5 (1) of the Directive of 1979, to take such steps as are necessary to ensure that it does not continue to be placed on the market, or, as may be appropriate, is not placed on the market until such authorised person is satisfied that the requirement is complied with.

REG 14

14. In addition to the powers conferred by Regulation 12 of these Regulations an authorised person may at all reasonable times enter an inspect any premises or any railway wagon, vehicle, ship, vessel or aircraft, or other thing used to transport (e.g. a container), in which he has reasonable grounds for believing that any feedingstuff is being marketed, manufactured for sale, imported, stored or kept for sale, sold or transported and may examine and take samples of any feedingstuff which he finds in the course of his inspection.

REG 15

15. (1) Where in any proceedings for an offence in which a contravention of these Regulation is alleged the defendant claims that the feedingstuff to which the alleged offence relates was exempted from these Regulations, by reason of paragraph (a) or (b) of Regulation 3 of these Regulations, the onus of proving that such feedingstuff was so exempted shall be on the defendant.
(2) In any proceedings for an offence under these Regulations, evidence of the result of any test, examination or analysis of, or of any report on, a sample taken under these Regulations may be

given if, and only if, it is proved that—
(a) the sample was taken and dealt with in accordance with the methods described in the Annex to Commission Directive No. 76/371/EEC of 1 March, 19761,
1 O.J. No. L102/1, 15 April 1976.
(b) before the proceedings were instituted one of the final samples prepared pursuant to the requirements of the said Annex was given or caused to be given to the defendant or sent or given to him by registered post or by such other method as stands approved of for the purposes of paragraph (b) of Regulation 11 (3) of these Regulations by the Minister, and
(c) the test, examination or analysis was carried out in accordance with such of the requirements (if any) specified in Part I of the Third Schedule to these Regulations as applied in the particular case.
(3) In any legal proceedings, other than proceedings to which paragraph (2) of this Regulation applies, evidence, being evidence which relates to an issue regarding the accuracy of a declaration required to be made by these Regulations, of the result of any test, examination or analysis of a sample of a feedingstuff may be given if, and only if, it is proved that—
(a) the sample was taken and dealt with in accordance with the methods referred to in paragraph (2) (a) of this Regulation,
(b) before the proceedings were instituted one of the final samples prepared pursuant to the requirements of the Annex to Commission Directive No. 76/371/EEC of 1 March, 1976, was given or caused to be given to the defendant, or sent or given to him by registered post or by any other method which is a method referred to in paragraph (2) (b) of this Regulation, and
(c) the test, examination or analysis was carried out in accordance with such of the requirements referred to in paragraph (2) (c) of this Regulation as applied in the particular case.

REG 16

16. The Minister shall furnish an authorised person with a certificate of his appointment and, when exercising any powers conferred by these Regulations, the authorised person shall if requested by any person affected, produce the certificate to that person.

REG 17

17. In any legal proceedings the production of a certificate in the form specified in Part II of the Third Schedule to these Regulations and purporting to be signed by the State Chemist shall, without proof of any signature on the certificate or that the signatory was the proper person to sign it, be sufficient evidence of the facts stated in the certificate and of the analysis to which it relates having been carried out in accordance with such of the requirements (if any) specified in Part I of the said Third Schedule as applied in the particular case.

REG 18

18. (1) Any person who—
(a) contravenes Regulation 5 or 6 of these Regulations,
(b) in marketing a feedingstuff—
(i) contravenes Regulation 9 of these Regulations,
(ii) fails to comply with a requirement of Regulation 8 or 10 of these Regulations, or of the said Regulation 8, as applied by Regulation 9 (3) of these Regulations, other than a requirement as regards the disclosed composition of a feedingstuff,
(c) contravenes any of the provisions of Regulation 12 of these Regulations,
(d) fails to comply with a requirement made of him under Regulation 13 of these Regulations,
(e) obstructs or interferes with an authorised person in the course of exercising a power conferred on him under Regulation 14 of these Regulations,
shall be guilty of an offence.
(2) Any person who in marketing a feedingstuff fails to comply with a requirement of Regulation 8 or 9 of these Regulations as regards the disclosed composition of a feedingstuff shall, subject to the limits of error specified in the Fourth Schedule to these Regulations, be guilty of an offence.
(3) If any person fraudulently—
(a) tampers with any thing so as to procure that any sample taken pursuant to Regulation 11 or 14 of these Regulations does not correctly represent the substance sampled, or
(b) tampers or interferes with any sample taken under these Regulations,
he shall be guilty of an offence.
(4) Any person guilty of an offence under these Regulations shall be liable on summary conviction to a fine not exceeding £500, or, at the discretion of the court, to imprisonment for a term not exceeding six months.
(5) An offence under these Regulations may be prosecuted by the Minister.

FIRST SCHEDULE

PART I
STRAIGHT FEEDINGSTUFFS

Name of FeedingstuffDescriptionCompulsory DeclarationsOptional DeclarationsCompositional Requirements1234561.Oil cakes and meals1.1Macoya palm kernel expeller.By-product of oil manufacture, obtained by pressing from seeds separated from their pulp of the following species of Macoya palm: Acrocomia sclerocarpa Mart, and Acrocomia totai Mart.Crude

protein
Crude fibre
Crude oil and fat
Crude ash
Moisture >12%Moisture <12%Ash insoluble in HCl Max 2%1.2Macoya extracted palm kernal.By-product of oil manufacture, obtained by extraction from seeds of Macoya palm separated from their pulp.Crude protein
Crude fibre
Crude ash
Moisture >12.5%Crude oil and fat Moisture <12.5%Ash insoluble in HCl Max. 2%1.3Macoya palm pulp.By-product of oil manufacture, obtained by pressing from pulp of Macoya palm.Crude protein
Crude fibre
Crude oil and fat
Crude ash
Moisture >12%Moisture <12%Ash insoluble in HCl Max. 2%1.4Decorticated ground nut expeller.By-product of oil manufacture, obtained by pressing from decorticated ground nuts(species Arachis hypogaea and other species of Arachis).Crude protein
Crude fibre
Crude oil and fat
Crude ash
Moisture >12%Moisture < 12%Ash insoluble in HCl Max. 2%1.5Extracted decorticated groundnut.By-product of oil manufacture, obtained by extraction from decorticated groundnut seeds.Crude protein
Crude fibre
Crude ash
Moisture >12.5%Crude oil and fat Moisture <12.5%Ash insoluble in HCl Max. 2%1.6Partly-decorticated groundnut expeller.By-product of oil manufacture, obtained by pressing from partly-decorticated ground nut seeds.Crude protein
Crude fibre
Crude oil and fat
Crude ash
Moisture >12%Moisture <12%Ash insoluble in HCl Max. 2%1.7Extracted partly-decorticated groundnut.By-product of oil manufacture, obtained by extraction from partly-decorticated ground nut seeds.Crude protein
Crude fibre
Crude ash
Moisture > 12.5%Crude oil and fat Moisture <12.5%Ash insoluble in HCl Max. 2%1.8Rape seed expeller.By-product of oil manufacture, obtained by pressing from seeds of colza Brassica napus L.ssp. oleifera (Metzg) Sinsk., of Indian sarson Brassica napus L. var. glauca (Roxb.) O.E. Schulz and of rape Brassica campestris L.ssp. oleifera (Metzg.) Sinsk.Crude protein
Crude fibre
Crude oil and fat
Crude ash
Moisture > 12%Moisture <12%Ash insoluble in HCl Max. 2%1.9Extracted rape seed.By-product of oil manufacture, obtained by extracting from seeds of colza, Indian sarson or rape.Crude protein
Crude fibre
Crude ash
Moisture > 12.5%Crude oil and fat Moisture <12.5%Ash insoluble in HCl Max. 2%1.10Copra expeller.By-product of oil manufacture, obtained by pressing from copra, the dried kernel (endosperm) and testa of the coconut palm, Cocos nucifera L.Crude protein
Crude fibre
Crude oil and fat
Crude ash

Moisture > 12%Crude oil and fat Moisture <12%Ash insoluble in HCl Max. 2%1.11Extracted copra.By-product of oil manufacture, obtained by extraction from copra, the dried kernel (endosperm) and testa of the coconut palm.Crude ash
Crude protein
Crude fibre
Moisture > 12.5%Crude oil and fat Moisture <12.5%Ash insoluble in HCl Max. 2%1.12Palm kernel expeller.By-product of oil manufacture, obtained by pressing from palm nuts, from which as much as possible of the hard shell has been removed, of the following species of oil palm: Elaeis guineensis Jacq., Corozo oleifera (H.B.K.) L. H. Bailey (Elaeis melanococca-auct.)Crude protein
Crude fibre
Crude oil and fat
Crude ash
Moisture > 12%Moisture <12%Ash insoluble in HCl Max. 2%1.13Extracted palm kernel.By-product of oil manufacture, obtained by extraction from palm nuts of the species of oil palm from which as much as possible of the hard shell has been removed.Crude protein
Crude fibre
Crude ash
Moisture > 12.5%Crude oil and fat

Moisture <12.5%Ash insoluble in HCl Max. 2%1.14Soya expeller.By-product of oil manufacture, obtained by pressing from soya beans (the seed of the species Glycine max. (L.) Merr.).Crude protein
Crude fibre
Crude oil and fat
Crude ash
Moisture > 12%Moisture <12%Ash insoluble in HCl Max. 2%1.15Extracted toasted soya.By-product of oil manufacture, obtained from soya bean seeds by extraction and appropriate heat treatment.Crude ash
Crude protein
Crude fibre
Moisture > 12.5% Crude oil and fat

Moisture <12.5%Ash insoluble in HCl Max. 2%1.16Extracted toasted hulled soya seeds.By-product of oil manufacture, obtained from hulled soya bean seeds by extraction and appropriate heat treatment.Crude protein
Crude fibre
Crude ash
Moisture > 12.5%Crude oil and fat

Moisture <12.5%Ash insoluble in HCl Max. 2%1.17Decorticated cotton seed expeller.By-product of oil manufacture, obtained by pressing from seeds of cotton belonging to the genus Gossypium spp. from which the fibres and husks have been removed.Crude protein

Crude fibre
Crude oil and fat
Crude ash
Moisture > 12%Moisture <12%Ash insoluble in HCl Max. 2%1.18Extracted decorticated cotton seed.By-product of oil manufacture, obtained by extraction from seeds of cotton from which the fibres have been removed.Crude protein
Crude fibre
Crude ash
Moisture > 12.5% Crude oil and fat

Moisture <12.5%Ash insoluble in HCl Max. 2%1.19Partly-decorticated cotton seed expeller.By-product of oil manufacture, obtained from seeds of cotton from which the fibres and part of the husks have been removed.Crude protein

Crude fibre
Crude oil and fat
Crude ash
Moisture > 12%Moisture <12%Ash insoluble in HCl Max. 2%1.20Extracted, partly-decorticated cotton seed.By-product of oil manufacture, obtained by extraction from seeds of cotton from which the fibres and part of the husks have been removed.Crude protein
Crude fibre
Crude ash
Moisture >12.5%Crude oil and fat

Moisture <12.5%Ash insoluble in HCl Max. 2%1.21Expeller or extracted niger seed.By-product of oil manufacture, obtained by presenting seeds of the niger plant Guizotia abyssinica (L.f.) Cass.Crude protein
Crude fibre
Crude oil and fat
Crude ash
Moisture > 12%Moisture <12%Ash insoluble in HCl Max. 3.4%1.22Decorticated sunflower seed expeller.By-product of oil manufacture, obtained by pressing from seeds of the sunflower Helianthus annuus L. from which as much as possible of the husk has been removed.Crude protein
Crude fibre
Crude oil and fat
Crude ash
Moisture > 12%Moisture <12%Ash insoluble in HCl Max. 2%1.23Extracted decorticated sunflower seed.By-product of manufacture, obtained by extraction from seeds of the sunflower from which the husks have been removed as far as possible.Crude protein
Crude fibre
Crude ash
Moisture > 12.5%Crude oil and fat

Moisture <12.5%Ash insoluble in HCl Max. 2%1.24Partly-decorticated sunflower seed expeller.By-product of oil manufacture, obtained by pressing from seeds of the sunflower from which part of the husks have been removed.Crude ash
Crude protein
Crude fibre
Crude oil and fat
Moisture > 12%Moisture <12%Ash insoluble in HCl Max. 2%1.25Extracted Partly-decorticated sunflower seed.By-product of oil manufacture, obtained by extraction from seeds of the sunflower from which part of the husks have been removed.Crude protein
Crude fibre
Crude ash
Moisture > 12.5%Crude oil and fat

Moisture <12.5%Ash insoluble in HCl Max. 2%1.26Linseed expeller.By-product of oil manufacture, obtained by pressing from linseed, Linum usitatissimum L.Crude protein
Crude fibre
Crude oil and fat
Crude ash
Moisture > 12%Moisture <12%Ash insoluble in HCl Max. 2%1.27Extracted linseed.By-product of oil manufacture, obtained by extraction from linseed.Crude protein
Crude fibre
Crude ash
Moisture > 12.5%Crude oil and fat

Moisture <12.5%Ash insoluble in HCl Max. 2%1.28Babassu palm nut expeller.By-product of oil manufacture, obtained by pressing from palm nuts, from which as much as possible of the hard shell

has been removed, of the Brazilian Babassu palms Orbignya oleifera Burr and other species of Orbignya.Crude protein
Crude fibre
Crude oil and fat
Crude ash
Moisture > 12%Moisture <12%Ash insoluble in HCl Max. 2%1.29Rice germ expeller.By-product of oil manufacture, obtained by pressing from germ of rice Oryza sativa L. to which parts of the endosperm and tegument still adhere.Crude protein
Crude fibre
Crude oil and fat
Crude ash
Moisture > 12%Moisture <12%Ash insoluble in HCl Max. 2%1.30Extracted brown rice germ.By-product of oil manufacture, obtained by extraction from germ of rice to which parts of the endosperm and tegument still adhere.Crude protein
Crude fibre
Crude ash
Moisture > 12.5%Crude oil and fat

Moisture <12.5%Ash insoluble in HCl Max. 2%1.31Sesame seed expeller.By-product of oil manufacture, obtained by pressing from seeds of the sesame plant, Sesamum indicum L.Crude protein
Crude fibre
Crude oil and fat
Crude ash
Moisture > 12%Moisture <12%Ash insoluble in HCl Max. 5%1.32Extracted sesame seed.By-product of oil manufacture, obtained by extraction from seeds of the sesame plant.Crude protein
Crude fibre
Crude ash
Moisture > 12.5%Crude oil and fat

Moisture <12.5%Ash insoluble in HCl Max. 5%1.33Extracted cocoa bean.By-product of oil manufacture, obtained by extraction from dried and roasted cocoa bean seeds Theobroma Cacao L. from which as much as possible of the husk has been removed.Crude protein
Crude fibre
Crude ash
Moisture > 12.5%Crude oil and fat

Moisture <12.5%Ash insoluble in HCl Max. 2%1.34Wheat germ expeller.By-product of oil manufacture, obtained by pressing from wheat germ of the species Triticum aestivum L., Triticum durum Desf. and from other cultivated species of husked wheat or from screened husked grains of spelt of the species Triticum spelta L., Triticum dicoccum Schrank, Triticum monococcum L., to which parts of the endosperm and tegument still adhere.Crude protein
Crude fibre
Crude oil and fat Crude ash
Moisture > 12%Moisture <12%Ash insoluble in HCl Max. 2%1.35Maize germ expeller (by-product of maize milling).By-product of oil manufacture, obtained by pressing and by a dry process, from maize germ Zea mays L. to which parts of the endosperm and testa still adhere.Crude protein
Crude fibre
Crude oil and fat Crude ash
Moisture > 12.5%Starch

Moisture <12.5%Ash insoluble in HCl Max. 2%1.36Extracted maize germ (by-product of maize milling).By-product of oil manufacture, obtained by extraction and by a dry process, from maize germ to which parts of the endosperm and testa still adhere.Crude protein
Crude fibre
Crude ash

Moisture > 12.5%Crude oil and fat Starch

Moisture <12.5%Ash insoluble in HCl Max. 2%1.37Maize germ expeller (by-product of the starch industry).By-product of oil manufacture, obtained by pressing and by a wet process, from maize germ to which parts of the endosperm and testa still adhere.Crude protein
Crude fibre
Crude oil and fat
Crude ash Moisture > 12.5%Moisture <12.5% Ash insoluble in HCl Max. 2%1.38Extracted maize germ (by-product of the starch industry).By-product of oil manufacture, obtained by extraction and by a wet process, from maize germ to which parts of the endosperm and testa still adhere.Crude protein
Crude fibre
Crude ash
Moisture > 12.5%Crude oil and fat

Moisture <12.5%Ash insoluble in HCl Max. 2%1.39Olive pulp meal.By-product of oil manufacture, obtained by extraction from fruits of the olive tree Olea Europa L. free as far as possible from fragments of stone.Crude protein
Crude fibre
Crude ash
Moisture > 12.5%Crude oil and fat

Moisture <12.5%Ash insoluble in HCl Max. 2%2.Products and by-products of the processing of vegetable substances2.1by-product of milling2.1.1Wheat bran.By-product of flour manufacture, obtained from screened husked grains of wheat or spelt. It consists principally of fragments of the outer skins, and of particles of grain from which the greater part of the endosperm has been removed.Crude fibre
Crude ash
Moisture > 14%Moisture <14%Ash insoluble in HCl Max. 2%2.1.2Wheat feed.By-product of flour manufacture, obtained from screened husked grains of wheat or spelt. It consists principally of fragments of the outer skins and of particles of grain from which less of the endosperm has been removed than in wheat bran.Crude fibre
Starch
Crude ash
Moisture > 14%Moisture <14%Ash insoluble in HCl Max. 2%2.1.3Wheat middlings.By-product of flour manufacture, obtained from screened husked wheat or spelt. It consists principally of particles of endosperm with fine fragments of the outer skins and some grain waste.Crude fibre
Starch
Crude ash
Moisture > 14%Moisture <14%Ash insoluble in HCl Max. 2%2.1.4Wheat germ.By-product of milling consisting essentially of wheat germ, rolled or otherwise, to which fragments of endosperm and outer skin still adhere.Crude fibre
Crude protein
Crude oil and fat Crude ash
Moisture > 12%Moisture <12%Ash insoluble in HCl Max. 2%2.1.5Rye bran.By-product of flour manufacture, obtained from screened rye secale cereale L. It consists principally of fragments of the outer skins, and of particles of grain from which most of the endosperm has been removed.Crude fibre
Crude ash
Moisture > 14%Moisture <14%Ash insoluble in HCl Max. 2%2.1.6Rye feed.By-product of flour manufacture, obtained from screened rye. It consists principally of fragments of the outer skins, and of particles of grain from which less of the endosperm has been removed than in rye bran.Crude fibre
Starch
Crude ash
Moisture > 14%Moisture <14%Ash insoluble in HCl Max. 2%2.1.7Rye screenings (rye meal).By-products of flour manufacture, obtained from screened rye. It consists principally of particles of endosperm, with fine fragments of the outer skins and some grain waste.Crude fibre

Starch
Crude ash
Moisture > 14%Moisture <14%Ash insoluble in HCl Max. 2%2.2Products and by-products of the manufacture of flakes, groats and husked grain.2.2.1Husked oat sharps (middlings).By-product, rich in starch, obtained during the processing of screened, husked, oats Avena sativa L. and other cultivated species of oats into oat groats or sifted oatmeal.Crude fibre
Starch
Crude ash
Moisture > 14%Moisture <14%Ash insoluble in HCl Max. 2%2.2.2Flaked barley.Product obtained by steaming and rolling husked barley Hordeum vulgare L.Crude fibre
Starch
Moisture > 14%Moisture <14%Ash insoluble in HCl Max. 0.5%2.2.3Barley feed.By-product of the processing of screened and husked barley into pearl barley or semolina or sifted barley meal.Crude fibre
Starch
Crude ash
Moisture >14%Moisture < 14%Ash insoluble in HCl Max. 2%2.2.4Flaked Maize.Product obtained by steaming and rolling maize.Crude fibre
Starch
Moisture > 14%Moisture <14%Ash insoluble in HCl Max. 0.5%2.2.5Pea middlings (pea forage meal).By-product obtained during the manufacture of pea-meal Pisum sativum L. It consists principally of particles of endosperm, and to a lesser extent, of skins.Crude fibre Crude oil and fat Crude protein Moisture > 14%Moisture <14%Ash insoluble in HCl Max. 2%2.2.6Flaked potatoes.Product obtained by drying potatoes, Solanum tuberosum L., whether or not peeled, which have been steamed or boiled and crushed.Crude fibre
Starch
Moisture > 14%Moisture <14%Ash insoluble in HCl Max. 1.7%2.3By-products of maize milling.2.3.1Maize feed meal.By-product of the manufacture of flour or semolina from maize.Starch
Crude protein
Crude oil and fat Crude fibre
Moisture > 14%Crude ash Moisture <14%Ash insoluble in HCl Max. 2%2.3.2Maize bran.By-product of the manufacture of flour or semolina from maize. It consists principally of outer skins and maize germ, with some endosperm particles.Crude fibre
Crude protein
Crude oil and fat
Moisture > 14%Crude ash

Moisture <14%Ash insoluble in HCl Max. 2%2.3.3Maize germ and bran.By-product of the manufacture of maize flour, maize semolina or of maize starch consisting of non-extracted germ, maize bran and some fragments of endosperm.Crude oil and fat Crude protein
Crude fibre
Moisture > 13%Crude ash
Starch

Moisture <13%Ash insoluble in HCl Max. 2%2.4Product and by-products of rice milling2.4.1Ground fodder rice.Product obtained by grinding fodder rice consisting either of green, chalky or unripe grains, sifted out during the milling of husked rice, or of normal husked grains which are yellow or spotted.Starch
Moisture > 14%Crude fibre
Crude ash
Crude oil and fat
Crude protein Moisture <14%Ash insoluble in HCl Max. 1%2.4.2Broken rice.By-product of the preparation of polished or glazed rice. It consists principally of under-sized or broken grains.Starch
Moisture > 14%Ash insoluble in HCl Max. 1%2.4.3Rice bran
(brown)By-product of the first polishing of husked rice. It consists

of silvery skins, particles of the aleurone layer, endosperm and
germ.Crude protein
Crude fibre
Crde oil and fat
Crude ash
Moisture > 12%Ash insoluble in HCl.

Moisture <12%Ash insoluble in HCl Max. 1.7%2.4.4Rice bran (white).By-product of the second polishing of husked rice. It consists principally of particles of endosperm, of the aleurone layer and of germ.Crude protein
Crude fibre
Crude oil and fat
Crude ash
Moisture > 12%Ash insoluble in HCl.

Moisture <12%Ash insoluble in HCl Max. 0.6%2.5Products and by-products of the starch industry.2.5.1Maize starchVirtually pure maize starch. Starch
Moisture > 14%Crude ash
Moisture <14%Ash insoluble in HCl Max. 2%2.5.2Puffed maize starch.Virtually pure maize starch, greatly expanded by appropriate heat treatment.Starch
Moisture > 10%Crude ash Moisture <10%Ash insoluble in HCl Max. 0.5%2.5.3Pre-gelatinized partially hydrolyzed maize starch.Virtually pure maize starch, largely pre-gelatinized and partially hydrolyzed. Starch
Reducing sugars expressed as glucose Moisture > 10%Crude ash Moisture <10%Ash insoluble in HCl Max. 0.5%2.5.4Maize gluten.Dried by-product of the manufacture of maize starch. It consists principally of gluten obtained during the separation of the starch.Crude protein
Crude oil and fat Moisture > 13%Crude fibre
Crude ash Xanthophyll Moisture <13%Ash insoluble in HCl Max. 0.5%2.5.5Maize gluten feedDried by-products of the manufacture of maize starch. It is composed of bran and of a smaller quantity of gluten. Dried residues of the steeping liquors, and germ, from which the oil has been removed, have been added.Crude protein
Crude fibre
Crude ash
Crude oil and fat Moisture > 13%Moisture <13%Ash insoluble in HCl Max. 2%2.5.6Rice starchVirtually pure rice starch Starch
Moisture > 14%Crude ash
Moisture <14%Ash insoluble in HCl Max. 0.5%2.5.7Puffed rice starchVirtually pure rice starch, greatly expanded by appropriate heat treatment.Starch
Moisture > 10%Crude ash
Moisture <10%Ash insoluble in HCl Max. 0.5%2.5.8Rice glutenDried by-product of the manufacture of rice starch, consisting mainly of gluten.Crude protein Moisture >13%Crude fibre
Crude ash
Crude oil & fat Moisture <13%Ash insoluble in HCl Max. 0.5%2.5.9Sorghum gluten feedDried by-product of the manufacture of sorghum starch Sorghum bicolor (L.) Moench s. 1. It consists of bran and a smaller quantity of gluten. Dried residues of the steeping liquors and the germ may be added.Crude protein
Crude oil & fat
Crude fibre
Crude ash
Moisture > 13%Moisture <13%Ash insoluble in HCl Max. 2%2.5.10Wheat starchVirtually pure wheat starch Starch
Moisture > 14%Crude ash
Moisture <14%Ash insoluble in HCl Max. 0.5%2.5.11Puffed wheat starchVirtually pure wheat starch greatly expanded by appropriate heat treatment.Starch

Moisture > 10%Crude ash Moisture <10%Ash insoluble in HCl Max. 0.5%2.5.12Pre-gelatinized, partially hydrolyzed wheat starchVirtually pure wheat starch, largely pregelatinized and partially hydrolyzed.Starch Reducing sugar expressed as glucose
Moisture > 10%Crude ash Moisture <10%Ash insoluble in HCl Max. 0.5%2.5.13Wheat glutenDried by-product of the manufacture of wheat starch. It consists principally of gluten obtained during the separation of starch.Crude protein Moisture >12%Crude ash Moisture <12%Ash insoluble in HCl Max. 0.5%2.5.14Manioc starchVirtually pure starch obtained from manioc roofs Manihot esculenta Crantz.Starch
Moisture > 15%Crude ash Moisture <15%Ash insoluble in HCl Max. 0.5%2.5.15Puffed manioc starchStarch obtained from manioc roots, greatly expanded by appropriate heat treatment.Starch
Moisture > 10%Crude ash Moisture <10%Ash insoluble in HCl Max. 0.5%2.5.16Potato starchVirtually pure potato starch.Starch
Moisture > 14%Crude ash Moisture <14%Ash insoluble in HCl Max. 0.5%2.5.17Pre-gelatinized potato starchVirtually pure potato starch, greatly expanded by appropriate heat treatment.Starch
Moisture > 10%Crude ash Moisture <10%Ash insoluble in HCl Max. 0.5%2.5.18Pre-gelatinized partially hydrolyzed potato starchVirtually pure potato starch, greatly expanded and partially hydrolyzed.Starch Reducing sugars expressed as glucose
Moisture > 10%Crude ash Moisture <10%Ash insoluble in HCl Max. 0.5%2.5.19Potato proteinDried by-product of starch manufacture composed mainly of protein substances obtained by the separation of starch.Crude protein Moisture >14%Crude ash
Crude oil & fat Crude fibre Moisture <14%Ash insoluble in HCl Max. 0.5%2.5.20Dried potato pulpDried By-product of the manufacture of potato starch.Starch
Moisture > 14%Crude ash
Crude oil & fat Crude fibre Moisture <14%Ash insoluble in HCl Max. 2%2.5.21Dextrose (glucose).Product of the saccharification of starch consisting of purified, crystallized glucose(with or without water of crystallization).Glucose
Moisture > 10%Moisture <10%Ash insoluble in HCl Max. 2%2.5.22Dextrose Molasses.By-product obtained during the crystallization of dextrose.Reducing sugars, expressed as glucose.Crude ash MoistureAsh insoluble in HCl Max. 2%2.6Products and by-products of sugar manufacture2.6.1Sugar (sucrose).Beet or cane sugar in solid form.SucroseCrude ashAsh insoluble in HCl Max. 2%2.6.2Dried sugar beet slices.Product obtained by drying slices of washed sugar beet Beta vulgaris L., ssp. vulgaris var. altissima Doell.Total sugar, expressed as sucrose Crude ash
Moisture > 13%Moisture <13%Ash insoluble in HCl Max. 2%2.6.3Dried partially extracted sugar beetProduct obtained by drying washed sugar beet slices.Total sugar, expressed as sucrose Crude ash
Moisture > 13%Moisture <13%Ash insoluble in HCl Max. 2%2.6.4Dried plain sugar beet pulpBy-product of the manufacture of sugar, consisting of pulped and dried sugar beet slices.Crude fibreAsh insoluble in HCl Max. 3.5%2.6.5Sugar beet molasses.By-product consisting of the syrupy residue collected during the manufacture or refining of beet sugar.Total sugar, expressed as sucroseAsh insoluble in HCl Max. 2%2.6.6Sugar cane molasses.By-product consisting of the syrupy residue collected during the manufacture of refining of sugar from sugar cane Saccharum officinarum L.Total sugar, expressed as sucroseAsh insoluble in HCl Max. 2%2.6.7Wet sugar beet pulp.By-product consisting of the undried residue resulting from the extraction of sugar from sugar beet.Crude protein
Crude oil
Crude fibre
Crude ash
MoistureAsh insoluble in HCl Max. 2%2.7Products and by-products of malting, brewing distilling and fruit processing; dried feed yeasts2.7.1Barley malt culms.By-product of malting, consisting of dried rootlets and shoots of germinated barley.Crude protein
Crude fibre
Crude ash
Moisture > 12.5%Moisture <12.5%Ash insoluble in HCl Max. 2%2.7.2Dried yeasts.Yeasts, whether or not mixed, belonging to the families Saccharomycetaceae, Endo-mycetaceae and Cryptococcaceae,

cultivated on the following substrates: beet or core juice or molasses, distillers' or yeast-makers' wash, whey, cereals and products derived from their processing, solutions from the hydrolysis of fibrous material, the cells of which have been killed by drying.Crude protein
Crude ash
Moisture > 10%Ash insoluble in HCl
Moisture <10%Ash insoluble in HCl Max. 1.1%2.7.3Dried brewers' grains.By-product of brewing obtained by drying residues of malted and un-malted cereals and other starchy matter.Crude protein
Crude fibre
Moisture > 12.5%Moisture <12.5%Ash insoluble in HCl Max. 2%2.7.4Dried distillers' grains.By-product of distilling obtained by drying residues of fermented cereals or other starchy matter.Crude protein
Crude fibre
Moisture > 12.5%Moisture <12.5%Ash insoluble in HCl Max. 2%2.7.5Dehydrated citrus pulp.By-product obtained during the manufacture of citrus juice.Crude fibre
Moisture > 13%Moisture <13%Ash insoluble in HCl Max. 2%2.7.6Wet brewers grains.By-product of brewing consisting of the undried residues of malted and unmalted cereals and other starchy matter.Crude protein Crude oil
Crude fibre
Crude ash
MoistureAsh insoluble in HCl Max. 2%.2.7.7Wet distillers grains.By-product of distilling consisting of undried residues of fermented cereals or other starchy matter.Crude protein Crude oil
Crude fibre
Crude ash MoistureAsh insoluble in HCl Max. 2%2.8Artificially dried agricultural products2.8.1Grass meal.Product obtained by artificially drying and possibly pre-drying young forage plants, the enzymes which activate oxidation being rendered virtually inactive by the drying.Crude protein
Crude fibre
Crude ash
Moisture > 12%Crude oil and fat Carotene Ash insoluble in HCl
Moisture <12%Ash insoluble in HCl Max. 3.4%2.8.2Lucerne meal.Product obtained by artificially drying and possibly pre-drying lucerne Medicago sativa L. and Medicago varia Martyn, the enzymes which active oxidation being rendered virtually inactive by the drying. This product may contain approximately 20% of grass or clover artificially dried and possibly pre-dried at the same time as the lucerne.Crude protein
Crude ash
Crude fibre
Moisture > 12%Crude oil and fat Carotene
Ash insoluble in HCl Moisture <12%Ash insoluble in HCl Max. 3.4%2.8.3Clover meal.Product obtained by artificially drying and possible pre-drying young clover Trifolium spp. the enzymes which activate oxidation being rendered virtually inactive by the drying. This product may contain approximately 20% of grass or lucerne artifically dried and possibly pre-dried at the same time as the clover.Crude protein
Crude ash
Crude fibre
Moisture > 12%Crude oil and fat Carotene Ash insoluble in HCl
Moisture <12%Ash insoluble in HCl Max. 3.4%2.8.4Dried tops and leaves of sugar beet.Product obtained by artificially drying tops and leaves of sugar beet, washed, whether or not chopped.Crude protein
Crude fibre
Total sugar, expressed as sucrose
Moisture > 12%Ash insoluble in HCl Moisture <12%Ash insoluble in HCl Max. 4%2.8.5Jerusalem artichoke chips or Jerusalem artichoke meal.Product obtained by crushing or grinding dried, cleaned tubers of Jerusalem artichokes Helianthus tuberosus L.Inulin

Crude fibre
Moisture > 13%Crude ash
Crude oil and fat Crude protein Moisture <13%Ash insoluble in HCl Max. 2%2.8.6Sweet potato chips or sweet potato meal.Product obtained by crushing or grinding dried, cleaned tubers of sweet potato Ipomoea batatas (L.) Poir.Starch
Moisture > 13%Crude ash
Crude fibre
Crude oil and fat
Crude protein
Moisture <13Ash insoluble in HCl Max. 2%2.8.7Manioc meal or manioc flakes or manioc roots.Dried and, if necessary, washed and peeled manioc roots; also products obtained by crushing and grinding.Starch
Crude ash
Moisture > 13%Crude fibre
Crude oil and fat
Crude protein
Moisture <13%Ash insoluble in HCl Max. 3.3%2.8.8Manioc meal type 55 or manioc flakes type 55 or manioc roots type 55.Unpeeled manioc roots, dried and, if necessary, washed; also products obtained by crushing and grinding.Starch
Crude ash
Moisture > 13%Crude fibre
Crude oil and fat
Crude protein
Moisture <13%Ash insoluble in HCl Max. 4%2.8.9Dried manioc pulpWaste from the manufacture of manioc starch, which has been dried and ground.Starch
Crude ash
Moisture > 13%Crude fibre
Crude oil and fat
Crude protein
Moisture <13%Ash insoluble in HCl Max. 2.3%2.9Other products of vegetable origin
2.9.1Crushed locust beansProduct obtained by crushing the dried,
stoned fruit of the carob tree Ceratonia siliqua L.Total sugar,
expressed as sucrose
Moisture > 14%Crude ash
Moisture <14%Ash insoluble in HCl Max. 2%2.9.2Vegetable fat or vegetable oilProduct composed of fat or oil of vegetable originMatter insoluble in light
petroleum > 1.5%
Acid lndex > 12 Moisture > 1%Matter insoluble in light petroleum
<1.5% Acid lndex<12 Moisture<1%Ash insoluble in HCl Max. 2%2.9.3BarleyWhole grain of cultivated varieties of barleyHectolitre weight Moisture >15%Crude fibre
Crude protein Crude oil
Crude ash
Lysine
Methionine Cystine
Starch Moisture <15%Ash insoluble in HCl Max. 2%2.9.4Processed barley other than flaked barley(2.2.2) (indicate type of process in name)Product obtained by the processing of whole barleyCrude fibre
Moisture > 14%Crude protein Crude oil
Crude ash
Lysine
Methionine Cystine
Starch
Moisture <14%Ash insoluble in HCl Max. 2%2.9.5OatsWhole grain of cultivated varieties of oats.Hectolitre weight Moisture >15%Crude fibre
Crude protein Crude oil

Crude ash
Lysine
Methionine Cystine
Starch
Moisture <15%Ash insoluble in HCl Max. 2%2.9.6Processed oats (Indicate type of process in name)Product obtained by the processing of whole oatsCrude fibre
Moisture > 14%Crude protein Crude oil
Crude ash
Starch
Lysine
Methionine
Cystine Moisture <14%Ash insoluble in HCl Max. 2%2.9.7WheatWhole grain of cultivated varieties of wheatHectolitre weight Moisture >15%Crude fibre
Crude protein Crude oil
Crude ash
Starch
Lysine
Methionine Cystine Moisture <15%Ash insoluble in HCl Max. 2%2.9.8Processed wheat(Indicate type of process in name)Product obtained by the processing of whole wheat.Crude fibre
Moisture > 14%Crude protein Crude oil
Crude ash
Starch
Lysine
Methionine
Cystine Moisture <14%Ash insoluble in HCl Max. 2%2.9.9MaizeWhole grain of cultivated varieties of maizeHectolitre weight Moisture >14%Crude fibre
Crude protein Crude oil
Crude ash
Starch
Lysine
Methionine
Cystine Moisture <14%Ash insoluble in HCl Max. 2%2.9.10Processed maize other than flaked maize (2.2.4) (Indicate type of process in name)Product obtained by the processing of whole maize.Moisture >13%Crude fibre
Crude protein Crude oil
Crude ash
Starch
Lysine
Methionine
Cystine Moisture <13%Ash insoluble in HCl Max. 2%2.9.11MiloWhole grain of cultivated varieties of milo.Hectolitre weight Moisture >14%Crude fibre
Crude protein Crude oil
Crude ash
Starch
Lysine
Methionine
Cystine Moisture <14%Ash insoluble in HCl Max. 2%2.9.12Processed milo (Indicate type of process in name)Product obtained by the processing of whole milo.Moisture >13%Crude fibre
Crude protein Crude oil
Crude ash
Starch
Lysine
Methionine
Cystine Moisture <13%Ash insoluble in HCl Max. 2%2.9.13PeasSeeds of the cultivated varieties of peasCrude protein Moisture >14%Crude oil

Crude fibre
Crude ash
Lysine
Methionine
Cystine Moisture <14%Ash insoluble in HCl Max. 2%2.9.14Processed peas (Indicate type of process in name)Product obtained by the processing of whole peasCrude protein Moisture >14%Crude oil
Crude fibre
Crude ash
Lysine
Methionine
Cystine Moisture <14%Ash insoluble in HCl Max. 2%2.9.15BeansSeeds of the cultivated varieties of beansCrude protein Moisture >14%Crude oil
Crude fibre
Crude ash
Lysine
Methionine
Cystine Moisture <14%Ash insoluble in HCl Max. 2%2.9.16Processed beans (Indicate type of process in name)Product obtained by the processing of whole beans.Crude protein Moisture >14%Crude oil
Crude fibre
Crude ash
Lysine
Methionine
Cystine
Moisture <14%Ash insoluble in HCl Max. 2%2.9.17Seaweed mealProduct obtained by the processing of seaweed.Crude ash
Moisture > 14%Crude protein Crude oil
Crude fibre Calcium Phosphorus Moisture <14%Ash insoluble in HCl Max. 2%2.9.18Rape seedSeeds of colza Brassica napus L. ssp. oleifera (Metzg.) Sinsk., of Indian sarson Brassica napus L. var. glauca (Roxb.) O.E Schulz and of rape Brassica campestvis L. ssp. oleifera (Metzg.) Sinsk Crude protein
Crude oil and fat Crude fibre
Crude ash
Moisture > 9%Moisture <9%Ash insoluble in HCl Max. 2%2.9.19Heat processed soya beansProduct obtained from heating whole soya beans without removing any of the component parts.Crude Protein
Crude oil and fat Crude fibre
Crude ash
Moisture > 12%Moisture <12%Ash insoluble in HCl Max. 2%3.Products of Animal Origin3.1Milk products3.1.1'Spray' skimmed-milk powder, 'hatmaker' or 'roller' skimmed-milk powderProduct obtained by drying skimmed milk either by vaporization in a current of hot air ('spray' skimmed milk powder) or by drying over cylinders ('hatmaker' or 'roller' skimmed milk).Crude protein
Lactose
Crude ash
Moisture > 5%Crude oil & fat
Moisture <5%Ash insoluble in HCl Max. 0.5%3.1.2Powdered buttermilkProduct obtained by drying buttermilk either by vaporization in a current of hot air ('spray' powdered buttermilk) or by drying over cylinders ('hatmaker' or 'roller' powdered buttermilk).Crude protein
Crude oil & fat Lactose
Crude ash
Moisture > 6%Moisture <6%Ash insoluble in HCl Max. 0.5%3.1.3Powdered whey or whey crumbsProducts obtained by drying whey.Crude protein
Lactose
Crude ash
Sodium
Moisture > 8%Crude oil and fat Chlorides, expressed as NaCl
Moisture <8%Ash insoluble in HCl Max. 0.5%3.1.4Low-sugar powdered wheyProduct obtained by drying whey from which the lactose has been partly extractedLactose

Crude protein
Crude ash
Sodium
Moisture > 8%Crude oil and fat
Chlorides, expressed as NaCl
Moisture <8%Ash insoluble in HCl Max. 0.5%3.1.5Powdered whey protein
Powdered milk albuminProducts obtained by drying the protein compounds extracted from whey or milk by chemical or physical treatment.Crude protein
Crude ash
Moisture > 8%Crude oil and fat
Moisture <8%Ash insoluble in HCl Max. 0.5%3.1.6Skim milkResidue obtained following the removal of fat from whole milk.Total solidsCrude protein Lactose
AshAsh insoluble in HCl Max. 2%3.1.7WheyBy-product obtained during the manufacture of cheese.Total solidsCrude protein Lactose
Ash
SodiumAsh insoluble in HCl Max. 2%3.1.8Concentrated wheyProduct obtained by removing part of the moisture from whey.Total solidsCrude Protein
Lactose
Ash
SodiumAsh insoluble in HCl Max. 2%3.2Products processed from land animals3.2.1Blood MealProduct obtained by drying the blood of slaughtered animals and poultry. This product should be substantially free of foreign matter.Crude Protein
Moisture > 10%Crude ash Moisture>10%Ash insoluble in HCl Max. 2%3.2.2Meat and bone mealProduct obtained by drying and grinding meat pieces containing a high proportion of bone from warm-blooded land animals. The product should be substantially free of hair, bristle, feathers, horn, hoof, skin and blood, and of the contents of the stomach and viscera. It must be technically free from organic solvents.Crude protein
Crude oil & fat
Total phosphorus Crude ash
Moisture > 10%Chlorides, expressed as NaCl Methionine
Lysine
Volatile nitrogenous bases
Moisture <10%Ash insoluble in HCl Max. 2%3.2.3Bone MealProduct obtained by drying and grinding bone, with the fat largely removed, from warm-blooded land animals. The product should be substantially free of hair, bristle, feathers, horn, hoof, skin and blood, and of the contents of the stomach and viscera. It should also be free of splinters and may not contain bone fragments with rough surfaces or jagged edges. It must be technically free from organic solvents.Crude protein
Total phosphorus
Crude ash
Crude oil & fat Moisture > 10%Moisture <10%Ash insoluble in HCl Max. 2%3.2.4Meat meal. Products with a fat content of more than 11% should be described as 'rich in fat'Product obtained by drying and grinding carcases and parts of carcases of warm-blooded land animals, if need be, with the fat removed by an appropriate process. It should be virtually free of hair, bristle, feathers, horn, hoof and skin, and the contents of the stomach and viscera. It shall be technically free of organic solvents.Crude protein
Crude oil & fat
Total phosphorus Moisture > 10%Chlorides, expressed as NaCl Ash insoluble in HCl
Methionine
Lysine

Volatile nitrogenous bases Moisture <10%Ash insoluble in HCl Max. 2.2%3.2.5GreavesProduct derived from residues of the manufacture of tallow and other fats of animal origin. It shall be technically free of organic solvents.Crude protein
Crude ash
Moisture > 10%Chlorides, expressed as NaCl Crude oil & fat Moisture <10%Ash insoluble in HCl Max. 0.5%3.2.6Dried waste from poultry slaughter. Products with a fat content of more than 12% should be described as 'rich in fat'.Product obtained by drying and grinding waste from slaughtered poultry; it should be substantially free of feathers.Crude protein
Crude oil & fat
Crude ash
Moisture > 10%Chlorides, expressed as NaCl
Moisture <10%Ash insoluble in HCl Max. 3.3%3.2.7Hydrolyzed feather meal.Product obtained by hydrolyzing, drying and grinding poultry feathers.Crude protein
Moisture > 11%Ash insoluble in HCl
Moisture <11%Ash insoluble in HCl Max 3.4%3.2.8Animal fatProduct composed of fat processed from warm-blooded land animals or from parts thereof. It shall be technically free of organic solvents.Acid Index >30 Matter insoluble in light
petroleum > 1.5% Moisture > 1%Acid index <30 Matter insoluble in light petroleum<1.5 Moisture<1%%Ash insoluble in HCl Max. 2%3.3Products derived from fish or other marine animals.3.3.1Fish meal (products whose chloride content expressed as NaCl is less than 2% may be referred to as 'low in salt')Product obtained by drying and grinding whole fish, or parts thereof, of various species. Concentrated press liquid may be added.Crude protein
Crude oil & fat Chlorides, expressed as NaCl
Total phosphorus Calcium carbonate Moisture > 10%Moisture <10%Ash insoluble in HCl Max. 2.2%3.3.2Cod liver oilOil obtained from fresh livers of fish of the cod family(Gadidae)Vitamin A Acid index > 6 Matter insoluble in light
petroleum > 0.05%
Moisture > 0.15%Acid index <6 Matter insoluble in light petroleum<0.05% Moisture>0.15%Ash insoluble in HCl Max. 2%4.MINERAL
SUBSTANCES4.1Calcium carbonate (the nature of the product(column 3)
should be indicated in the name)Precipitated calcium carbonate, ground
limestone, prepared chalk, granulated chalk, ground oyster or mussel
shells.Calcium
Ash insoluble in HClAsh insoluble in HCl Max. 5%4.2Calcium and
magnesium carbonateNatural mixture of calcium carbonate and magnesium
carbonate.Calcium
MagnesiumAsh insoluble in HCl Max. 2%4.3Calcareous marine algae
(Maerl)Product of natural origin obtained from calcareous algae,
ground or granulated.Calcium
Ash insoluble in HClAsh insoluble in HCl Max. 5%4.4Magnesium
oxideTechnically pure magnesium oxide MgOMagnesiumAsh insoluble in HCl Max. 2%4.5KieseriteNatural magnesium sulphateMgSO4H2OMagnesiumAsh insoluble in HCl Max. 2%4.6Calcium monohydrogen phosphate (dicalcium phosphate) (the manufacturing process may be indicated in the name)Technically pure calcium monohydrogen phosphate (dicalcium phosphate).Total phosphorus Chlorides, expressed as NaCl
CalciumAsh insoluble in HCl Max. 2%4.7Defluorinated natural
phosphateProduct obtained by grinding natural phosphates, purified and
defluorinated to a greater or lesser degree.Total phosphorus
CalciumAsh insoluble in HCl Max. 2%4.8De-gelatinized bone
mealDe-gelatinized, sterilized, ground bones from which the fat has
been removed.Total phosphorus
Calcium

Moisture > 10%Moisture <10%Ash insoluble in HCl Max. 2%4.9Calcium bis- (dihydrogen phosphate) (monocalcium phosphate)Product consisting principally of technically pure calcium bis-(dihydrogen phosphate) (monocalcium phosphate)Total phosphorus CalciumAsh insoluble in HCl Max. 2%4.10Ammonium dihydrogen phosphate (monoammonium phosphate)Product consisting mainly of technically pure ammonium dihydrogen phosphate.Total phosphorus NitrogenAsh insoluble in HCl Max. 2%4.11SaltTechnically pure sodium chloride (NaCl)Chlorides, expressed as NaClAsh insoluble in HCl Max. 2%4.12Other mineral substances except trace elements Common name to be used.Calcium > 5% Phosphorus > 5% Magnesium > 5% Sodium > 5%Calcium <5% Phosphorus<5% Magnesium<5% Sodium<5%Ash insoluble in HCl Max. 2%5OTHER STRAIGHT FEEDINGSTUFFS5.1Common name to be used.All feed ingredients not listed in Part I of this Schedule except those listed in Part II.Crude protein >5% Crude oil & fat > 2.5%
Crude fibre > 5% Crude ash > 4% Starch > 15% Moisture > 14%Crude protein <5% Crude oil & fat<2.5%
Crude fibre <5% Crude ash<4% Starch<15% Moisture<14%Ash insoluble in HCl Max. 2%
PART II
PROHIBITED STRAIGHT FEEDINGSTUFFS
Bagasse including molassed bagasse
Cocoa shells
Corozo nut meal
Dried poultry manure
Grape by-products from wine manufacture (grape pulp, grape follicle meal etc.)
Grass seed cleanings
Hoof or horn meal
Rice hulls
Shea nut meal or karite nut meal
Sal seed meal.
PART III
COMPOUND FEEDINGSTUFFS
(Contents of analytical constituents to be declared as a percentage of the weight of the compound feedingstuff as such)

(1)(2)(3)Type of compound feedingstuffsCompulsory declaration (Regulation 8(1) (b) (vi))Optional declarations (Regulation 9(2) (d))Compound—crude protein—soluble proteinfeedingstuffs with—crude oils and fats—starchthe exception of—crude fibre—total sugar, expressed as wholegrain mixes,—moisture where it sucrosemineral and equals or—total sugar plus starchmolassed exceeds 14%—moisturewhere the levels are less than those specified in column 2feedingstuffs and—crude ash—Calciumcompound—calcium, where it—magnesiumfeedingstuffs for petequals or—sodiumanimalsexceeds 5%—phosphorus-—magnesium,where it equals or exceeds 0.4%—sodium, where it—lysinein feeds for pigs, poultry or preruminating ruminants if not required in column 2 oppositeequals or exceeds—methionine0.5%—cystine—phosphorus, where it equals orexceeds 1%—lysine, in compoundfeedingstuffs for swine—methionine in compoundfeedingstuffs for poultry —cystine in compound feedingstuffs for poultryMineral feedingstuffs—calcium—crude ash—phosphorus—crude protein—sodium—crude oils and fats—magnesium—crude fibre—moisture content if it equals or exceeds 5% in mineral feedingstuffs containing no organic substances or 10% in mineral feedingstuffs containing organic substances—moisture where the levels are less than those specified in column 2 opposite—lysine, in mineral feedingstuffs for pigs—methionine

in mineral feedingstuffs for poultry—cystine in mineral feedingstuffs for poultryMolassed feedingstuffs—crude protein—soluble protein—crude oils and fats—starch—crude fibre—Total sugar plus starch—moisture, where it equals or exceeds 14%—moisture, where it is less than 14%—crude ash—calcium—total sugar expressed as sucrose—magnesium —sodium—phosphorus—lysine —methionine —cystinein feeds for pigs, poultry or preruminating ruminantsCompound feedingstuffs for cats and dogs—Crude protein —crude oils and fats—calcium —sodium—crude fibre—phosphorus—crude ash—moistureCompound feedingstuffs for pet animals other than cats and dogs—moisture —crude protein—crude oils and fats—crude fibre—crude ash—calcium—sodium—phosphorus

SECOND SCHEDULE

Fees Referred to in Regulation 11 (2) (b).

IR£Moisture4Crude protein6Soluble Protein15Volatile nitrogenous bases15Amino acids25 for one amino acid plus 2 for each addition al amino acidCrude oils and fats6Acid index10Matter insoluble in light petroleum10Crude fibre6Starch10Total sugars10Reducing sugars10Lactose10Glucose10Total solids5Skim milk powder15Crude ash3Ash soluble in HCl5Calcium carbonate10Chlorides expressed as NaCl5Calcium10Phosphorus10Sodium10Potassium10Magnesium10Bushel Weight4Vitamin A15

THIRD SCHEDULE

PART I

1. GENERAL PROVISIONS ON METHODS OF ANALYSIS FOR FEEDINGSTUFFS.
2. MOISTURE.
2.1 Determination of moisture.
2.2 Determination of moisture in oilseeds and oleaginous fruit.
2.3 Determination of moisture in milk products.
2.4 Determination of moisture in animal and vegetable fats and oils.
3. CARBOHYDRATES.
3.1 Determination of crude fibre
3.2 Determination of starch
3.2.1 Polarimetric method
3.2.2 Pancreatic method.
3.3 Determination of sugar
3.4 Determination of lactose.

1. GENERAL PROVISIONS ON METHODS OF ANALYSIS FOR FEEDINGSTUFFS.

A. PREPARATION OF SAMPLES FOR ANALYSIS.

1. Purpose

The procedures described below concern the preparation for analysis of final samples, sent to the control laboratories after sampling in accordance with the provisions laid down by First Commission Directive 76/371/EEC of 1 March 1976 establishing Community methods of sampling for the official control of feedingstuffs1.

1 OJ No. L 102, 15 April 1976, p.1.

These samples must be prepared in such a way that the amounts weighed out, as provided for in the methods of analysis, are homogeneous and representative of the final samples.

2. Precautions to be taken

All the necessary operations must be performed in such a way as to avoid as far as possible contamination of the sample and changes of its composition. Grinding, mixing and sieving should be carried out as quickly as possible with minimal exposure of the sample to the air and light. Mills and grinders likely to appreciably heat the sample should not be used. Manual grinding is recommended for feedingstuffs which are particularly sensitive to heat. Care should

also be taken to ensure that the apparatus itself is not a source of contamination of trace elements.
If the preparation cannot be carried out without significant changes in the moisture content of the sample, determine the moisture content before and after preparation according to the method laid down in method 2.1 of these regulations.
3. Procedure
Mix thoroughly the final sample either mechanically or manually.
Divide the sample into two equal portions (the quartering method should be used where applicable). Keep one of the portions in a suitable clean, dry container, fitted with an air-tight stopper, and prepare the other portion or a representative part of it, of at least 100g, as indicated below.
3.1 Feedingstuffs which can be ground as such.
Unless otherwise specified in the methods of analysis, sieve the whole sample through a sieve with a square mesh of 1 mm size (in accordance with recommendation ISO R565) after grinding, if necessary. Avoid any overgrinding.
Mix the sieved sample and collect it in a suitable clean dry container fitted with an air-tight stopper. Mix again, immediately before weighing out the amount for analysis.
Feedingstuffs which can be ground after drying.
Unless otherwise specified in the methods of analysis, dry the sample to bring its moisture content down to a level of 8 to 12% according to the preliminary drying procedure described under point 4.3 of the method of determination of moisture mentioned in section 2 above. Then proceed as indicated in section 3.1.
3.3 Liquid or semi-liquid feedingstuffs.
Collect the sample in a suitable clean, dry container, fitted with an air-tight stopper. Mix thoroughly immediately before weighing out the amount for analysis.
3.4 Other feedingstuffs.
Samples which cannot be prepared according to one of the above procedures should be treated by any other procedure which ensures that the amounts weighed out for the analysis are homogeneous and representative of the final samples.
4. Storage of samples
Samples must be stored at a temperature that will not alter their composition. Samples intended for the analysis of vitamins or substances which are particularly sensitive to light should be stored in brown glass containers.
B. PROVISIONS RELATING TO REAGENTS AND APPARATUS USED IN METHODS OF ANALYSIS.
1. Unless otherwise specified in the methods of analysis, all analytical reagents must be analytically pure (a.p.). When determining trace elements the purity of the reagents must be checked by a blank test. Depending upon the results obtained, further purification of the reagents may be required.
2. Any operation involving preparation of solutions, dilution, rinsing or washing, mentioned in the methods of analysis without indication as to the nature of the solvent or dilutent employed, implies that water must be used. As a general rule, water should be demineralized or distilled. In particular cases, which are indicated in the methods of analysis, it must be submitted to special procedures of purification.

3. In view of the equipment normally found in control laboratories, only those instruments and apparatus which are special or require specific usage are referred to in the methods of analysis. They must be clean, especially when very small amounts of substances have to be determined.

C. APPLICATION OF METHODS OF ANALYSIS AND EXPRESSION OF THE RESULTS.

1. In general a single method of analysis is established for the determination of each substance in feedingstuffs. Where several methods are given, the particular method used by the control laboratory must be indicated on the analysis report.

2. The result given in the analysis report shall be the average value obtained from at least two determinations, carried out on separate portions of the sample, and of satisfactory repeatability. This result shall be expressed in the manner laid down in the method of analysis to an appropriate number of significant figures and shall be corrected, if necessary, to the moisture content of the final sample prior to preparation.

2. MOISTURE.

2.1 DETERMINATION OF MOISTURE.

1. Purpose and scope

To determine the moisture content of feedingstuffs. 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/EEC1 of 22 September 1966 on the establishment of a common organisation of the market in oils and fats.

1 OJ No. 127. 30 September 1966. P. 3025/66.

2. Principle

The sample is desiccated under specified conditions which vary according to the nature of the feeding stuffs. The loss in mass is determined by weighing. It is necessary to carry out preliminary drying when dealing with solid feeding stuffs 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/cm2

3.4 Electrically heated isothermal oven (+1°C) properly ventilated and ensuring rapid temperature regulation.2

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 50g 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 50g of the sample. Grind into particles of which at least 50 per cent will pass through a 0.5 mm mesh sieve and will leave no more than 10 per cent reject on a 1 mm round-meshed sieve.

4.1.3 Feedingstuffs in liquid or paste form, feeding stuffs predominantly composed of oil.

Weigh, to the nearest 10 mg. approximately 25 g of the sample. 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, to the nearest 0.5 mg, a container (3.3) with its lid.

Weigh into the weighed container, to the nearest mg, about 5 g of the sample and spread evenly. Place the container, without its lid, in the oven preheated to 103°C. To prevent the oven temperature from falling unduly introduce the container as rapidly as possible. Leave to dry for 4 hours reckoned from the time when the oven temperature returns to 103°C. Replace the lid on the container, remove the latter from the oven, leave to cool for 30-45 minutes in the desiccator (3.6) and weigh to the nearest mg.

For feeding stuffs composed predominantly of oil, dry in the oven for an additional 30 minutes at 130°C. Cool in a desiccator and weigh. The difference between the two weighings must not exceed 0.1 per cent of moisture.

2For the drying of cereals, flour, groats and meal, the oven must have a thermal capacity such that when pre-set at 131°C it will return to that temperature in less than 45 minutes after the maximum number of lest 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 2 hours, the results differ from those obtained after 4 hours of drying by less than 0.15%.

4.2.2 Cereals, flour, groats and meal.

Weigh, to the nearest 0.5 mg, a container (3.3) with its lid.

Weigh into the weighed container, to the nearest mg, about 5 g of the crushed sample and spread evenly. Place the container, without its lid in the oven (3.4) preheated to 130°C. To prevent the oven temperature from falling unduly, introduce the container as rapidly as possible. Leave to dry for 2 hours reckoned from the time when the oven temperature returns to 130°C. Replace the lid on the container, remove the latter from the oven, leave to cool for 30-45 minutes in the desiccator (3.6) and weigh to the nearest mg.

4.2.3 Compound feedingstuffs containing more than 4 per cent of sucrose or lactose; straight feeding stuffs such as locust beans, hydrolized cereal products, maltculms, dried beet pulp, fish and sugar solubles; compound feeding stuffs containing more than 25 per cent of mineral salts including water of crystallisation.

Weigh, to the nearest 0.5 mg, a container (3.3) with its lid.

Weigh into the weighed container, to the nearest 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 80-85°C. 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 4 hours at this pressure, either in a current of hot, dry air or using a drying agent (about 300g 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 80-85°C. 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 30-45 minutes in the desiccator (3.6) and weigh to the nearest mg. Dry for an additional 30 minutes in the vacuum oven at 80-85°C and reweigh. The difference between the two weighings must not exceed 0.1 per cent of moisture.
4.3 Preliminary drying
4.3.1 Feeding stuffs 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, to the nearest 10 mg, approximately 50 g of uncrushed sample (compressed or agglomerated feeding stuffs may be roughly divided if necessary) in a suitable container (e.g. a 20x12 cm aluminium plate with a 0.5 cm rim). Leave to dry in an oven from 60-70°C until the moisture content has been reduced to between 8-12 per cent. Remove from the oven, leave to cool uncovered in the laboratory for 1 hour and weigh to the nearest 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 feeding stuff.
4.3.2 Cereals.
Grain with a moisture content of over 17 per cent must be subjected to preliminary drying as follows:
Weigh, to the nearest 10 mg, 50 g of unground grain in a suitable container (e.g. a 20x12 cm aluminium plate with a 0.5 cm rim). Leave to dry for 5-7 minutes in an oven at 130°C. Remove from the oven, leave to cool uncovered in the laboratory for 2 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

where: E=initial mass, in grams, of the test sample;
m=mass, in gramms, of the dry test sample.
5.2 Drying with preliminary drying

where:E=initial mass, in grams, of the test sample;M=mass, in grams, of the test sample after preliminary drying;M1=mass, in grams, of the test sample after crushing or grinding;m=mass, in grams, of the dry test sample.
6. Repeatability
The difference between the results of two parallel determinations carried out on the same sample should not exceed 0.2% in absolute

value.
7. 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 feeding stuff must be corrected on the basis of the moisture content of the sample in its initial state.

2.2 DETERMINATION OF MOISTURE IN OILSEEDS AND OLEAGINOUS FRUIT.

1. Purpose and Scope
To determine the moisture content and volatile matter in oil seeds and oleaginous fruit.
2. Principle
The sample is dried at 103°C ± 2°C. The loss in mass is determined by weighing.
3. Apparatus
3.1 Analytical balance.
3.2 Flat bottomed containers of non-corrodible metal with lids ensuring airtight closure, and allowing the test sample to be spread to about 0.2g/cm².
3.3 Electrically heated isothermal oven 103°C ± 2°C properly ventilated and ensuring rapid temperature regulation.
4. Procedure
4.1 Preparation
4.1.1 Copra.
Grate the sample wither by hand or mechanically and ensure representativity. The length of the particles may exceed 2 mm but should not be greater than 5 mm. Mix the particles carefully and carry out the determination without delay.
4.1.2 Seeds of medium size (e.g. groundnut, soya etc.) except sunflower seed and cotton seed with adherent linters.
Grind the sample in a mechanical mill fitted with a 2 mm sieve. Mix and carry out the determination without delay.
4.1.3 Small seeds (e.g. linseed, colza, hemp etc.) as well as safflower seed, sunflower seed and cotton seed with adherent linters. These are analysed without previous grinding.
4.2 Drying.
Weigh to the nearest mg, a container (3.2) previously dried for 30 mins at 103°C ± 2°C and cooled in a desiccator.
Weigh into the container and to the nearest mg, 5 g±0.5 g of products coming under heading 4.1.1 and 4.1.2 and 5 to 10 g for products coming under heading 4.1.3.
Spread the material evenly over the whole base of the vessel and immediately place in the oven (3.3) previously heated to 103°C ± 2°C. Leave to dry for 3 hours (12 to sixteen hours for cotton seed with adherent linters) reckoned from the time the oven temperature returns to 103°C. Replace the lid on the container, remove from the oven, cool in a desiccator and weigh.
Return the uncovered container and lid to the oven for a further hour.
Replace the lid on the container, remove from the oven, cool in a desiccator and weigh.
Repeat the one hour drying operations until the difference between two successive weighings is equal to or less than 5 mg.
5. Calculation of results
The moisture content, as a percentage of the sample, is calculated by using the following formula.

where:E=initial mass in grams, of the test sample.m=mass in grams of the dry test sample.

6. Repeatability.

The difference between the results of two parallel determinations carried out on the same sample should not exceed 0.2% in absolute value.

2.3 DETERMINATION OF MOISTURE IN MILK PRODUCTS.

1. Purposes and scope

To determine the moisture content of milk products as straight feeding stuffs.

2. Principle

The sample is dried at 102°C ± 1°C. The loss in mass is determined by weighing.

3. Apparatus

3.1 Analytical balance.

3.2 Flat bottomed dishes of non-corrodible metal or of glass with lids ensuring airtight closure; Suitable dimensions are: diameter 60 to 80 mm and depth about 25 mm.

3.3 Electrically heated isothermal oven (±1°C) properly ventilated and ensuring rapid temperature regulation.

3.4 Desiccator, containing an efficient drying agent.

4. Procedure

Place dishes (3.2) in drying oven at 102°C (3.3) for approximately 60 minutes. Cool in a desiccator (3.4) and weigh accurately. Weigh to the nearest mg, approximately 2 g of sample into the dish. Transfer to oven (102°C) and leave sample uncovered for 2 hours. Replace the lid, transfer the covered dish to the desiccator, allow it to cool to room temperature and accurately weigh to the nearest 0.1 mg as quickly as possible.

Uncover the dish and heat it and its lid for one hour in the oven.

5. Calculation of results

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

where:E=initial mass, in grams, of the test sample;m=mass, in grams, of the dry test sample.

2.4 DETERMINATION OF MOISTURE IN ANIMAL AND VEGETABLE FATS AND OILS.

1. Purpose and scope

To determine the moisture and volatile substances content of animal and vegetable fats and oils.

2. Principle

The sample is dried to constant weight at 103°C. The loss in mass is determined by weighing.

3. Apparatus

3.1 Flat-bottomed dish, of a corrosion-resistant material, 8-9 cm in diameter and approximately 3 cm high.

3.2 Mercury thermometer with a strengthened bulb and expansion tube at the top end, graduated from approximately 80°C to at least 110°C, and approximately 10 cm in length.

3.3 Sand bath or electric hot-plate.

3.4 Desiccator, containing an efficient drying agent.

3.5 Analytical balance.

4. Procedure

Weigh, to the nearest mg, approximately 20 g of the homogenized sample into the dry, weighed dish (3.1) containing the thermometer (3.2). Heat on the sand bath or hot-plate (3.3), stirring continuously with the thermometer, so that the temperature reaches 90°C in about 7 minutes.
Reduce the heat, watching the frequency with which bubbles rise from the bottom of the dish. The temperature must not exceed 105°C. Continue to stir, scraping the bottom of the dish, until bubbles stop forming.
In order to ensure complete elimination of moisture, reheat several times to 103°C ± 2°C, cooling to 93°C between successive heatings. Then leave to cool to room temperature in the desiccator (3.4) and weigh. Repeat this operation until the loss in mass between two successive weighings no longer exceeds 2 mg.
N.B. An increase in the mass of the sample after repeated heating indicates an oxidation of the fat, in which case calculate the result from the weighing carried out immediately before the mass began to increase.
5. Calculation of results
The moisture content, as a percentage of the sample, is given by the following formula:

where:M0=mass, in grams, of the test sample;M1=mass. in grams, of the dish with its contents before heating;M2=mass, in grams, of the dish with its contents after heating.Results lower than 0.05% must be recorded as 'lower than 0.05%'.
6. Repeatability
The difference in moisture between the results of two parallel determinations carried out on the same sample must not exceed 0.05%, in absolute value.
3. CARBOHYDRATES.
3.1 DETERMINATION OF CRUDE FIBRE.
1. Purpose and scope
To determine, in feeding stuffs, the content of fat-free organic substances which are insoluble in acid and alkaline media and are conventionally described as crude fibre.
2. Principle
The sample, defatted where necessary, is treated successively with boiling solutions of sulphuric acid and potassium hydroxide of specified concentrations. The residue is separated by filtration in the presence of asbestos, washed, dried, weighed and ashed at 900°C. The loss of weight resulting from ashing corresponds to the crude fibre present in the test sample.
3. Reagents
3.1 Sulphuric acid, 0.26 N.
3.2 Treated asbestos: add to asbestos of the type used with a Gooch crucible approximately 5 times its weight of dilute hydrochloric acid (1 volume hydrochloric acid, d: 1.19+3 volumes of water). Boil the mixture for approximately 45 minutes, leave to cool and filter through a Buchner funnel. Wash the residue first with water until the washing water is free from acid, and then with acetone (3.6). Dry the asbestos in the drying oven and then ash for 2 hours at 900°C. Leave to cool and keep in a stoppered flask. Asbestos treated in this way may be used several times. It must meet the specifications given in 5 regarding the blank test.

3.3 Antifoam (e.g. silicone).
3.4 Potassium hydroxide solution 0.23 N.
3.5 Hydrochloric acid 0.5 N.
3.6 Acetone.
3.7 Light petroleum, boiling range 40-60°C.
4. Apparatus
4.1 Beakers of at least 600 ml capacity, with measuring marks at the 200 ml level.
4.2 Porcelain discs approximately 80 mm in diameter and approximately 4mm thick, perforated with approximately 32 holes, each approximately 4 mm in diameter.
4.3 Rubber-stoppered vacuum flasks of approximately 2 litre capacity, with measuring marks at the 800 ml level and fitted with glass funnels 120 mm in diameter.
4.4 Filter plates approximately 40 mm in diameter and approximately 4 mm thick, with slanting edges to fit the cone of the funnel (4.3), perforated with approximately 16 holes, each approximately 4 mm in diameter, and covered by a wire mesh, the mesh size being approximately 1 mm. Both plates and wire mesh must be resistant to acids and alkali.
4.5 Platinum or silica ashing crucibles.
4.6 Thermostatically controlled electric muffle-furnace.
4.7 Desiccator.
4.8 Asbestos filter: suspend 2.0 g asbestos (3.2) in 100 ml water. Filter under vacuum over the filter plate covered with a wire mesh (4.4) and placed in the funnel of a vacuum flask (4.3). Collect the filtrate and filter once more through the same filter. Discard the filtrate.
5. Procedure
Weigh, to the nearest mg approximately 3 g of the sample and 2 g treated asbestos (3.2) into a beaker (4.1), add 200 ml sulphuric acid (3.1) and a few drops of antifoam (3.3). Bring rapidly to the boil and leave to boil for exactly 30 minutes. To keep a constant volume, cover the beaker with a cooling device such as a 500 ml round-bottomed flask in which cold water is circulated. Stop boiling by adding approximately 50 ml cold water and filter immediately under vacuum through an asbestos filter previously prepared as shown in 4.8.
Wash the residue with 5 lots of approximately 100 ml of very hot water to obtain a final volume of filtrate of 800 ml. Transfer the residue quantitatively to the beaker (4.1) which has first been fitted with a porcelain disc (4.2) to regulate the boiling. Add 200 ml potassium hydroxide solution (3.4). Bring rapidly to the boil and leave to boil for exactly 30 minutes. Add approximately 50 ml cold water and filter immediately under vacuum through a fresh asbestos filter previously prepared as shown in 4.8. Wash the residue with very hot water until the washing water is neutral (test with litmus paper), then 3 times with acetone (3.6) (approximately 100 ml acetone in all).
Transfer the residue quantitatively to an ashing crucible (4.5), break up if necessary and dry to constant weight in the drying oven at 130°C.
Leave to cool in the desiccator (4.7) and weigh rapidly.
Place the crucible in the muffle-furnace (4.6) and leave to ash for 30 minutes at 900°C. Leave to cool in the desiccator (4.7) and

weigh rapidly.
Carry out a blank test applying the same procedure to the treated asbestos (3.2), but without the sample. Loss of weight resulting from ashing of the 6 g asbestos must not exceed 10 mg.
6. Calculation of results
The crude fibre content, as a percentage of the sample, is given by the formula:

where:
a=loss of weight after ashing during the determination;
b=loss of weight after ashing during the blank test.
7. Repeatability
The difference between the results of two parallel determinations carried out on the same sample must not exceed:
0.3%, in absolute value, for crude fibre contents less than 10%;
3%, relative to the higher result, for crude fibre contents equal to or greater than 10%.
8. Observations
8.1 Feeding stuffs containing more than 10% oil must be defatted prior to analysis with light petroleum (3.7). To do this, place the test sample (3 g weighed to the nearest mg) on an asbestos filter (4.8). Cover 3 times with approximately 50 ml light petroleum (3.7) and each time filter carefully under vacuum. Transfer the defatted test sample and the asbestos quantitatively to a beaker (4.1) and continue the analysis as shown in 5.
8.2 Feeding stuffs containing oil which cannot be extracted directly must be defatted as shown in 7.1 and defatted a further time after the acid attack has been washed from the residue.
To do this, wash the residue 3 times with acetone (3.6) (100 ml in all), then 3 times with 50 ml light petroleum (3.7). Then transfer the residue quantitatively to a beaker (4.1) and continue the analysis as shown in the second paragraph of 5 (treatment with potassium hydroxide solution).
8.3 If the feeding stuffs are rich in calcium (more than 2% calcium), place the test sample (3 g, weighed to the nearest mg) in a beaker (4.1) with 100 ml hydrochloric acid 0.5 N (3.5) and leave to stand in a cool temperature for 5 minutes. Filter immediately and wash in cold water. Use as a filtration aid the 2.0 g asbestos specified for boiling with sulphuric acid. If filtration proves difficult, dilute the suspension with acetone (3.6). Then proceed as shown in 5.
3.2 DETERMINATION OF STARCH.
3.2.1 Polarimetric method
1. Purpose and scope
To determine the levels of starch and of high molecular weight starch degradation products in feedingstuffs, with the exception of those feedingstuffs which contain beet chips, beet pulp, dried beet tops or leaves, potato pulp, dehydrated yeast, products rich in inulin (e.g. chips and meal of Jerusalem artichokes) or greaves.
2. Principle
The method comprises two determinations. In the first, the sample is treated when hot with dilute hydrochloric acid. After clarification and filtration the optical rotation of the solution is measured by polarimetry.
In the second, the sample is extracted with 40% ethanol. After

acidifying the filtrate with hydrochloric acid, clarifying and filtering, the optical rotation is measured as in the first determination.

The difference between the two measurements, multiplied by a known factor, gives the starch content of the sample.

3. Reagents

3.1 Hydrochloric acid, d : 1.126.

3.2 Hydrochloric acid solution, 1.128% (w/v).

The concentration must be checked by titration using a sodium hydroxide solution 0.1 N in the presence of 0.1% (w/v) methyl red in 94% (v/v) ethanol. 10 ml=30.94 ml of NaOH 0.1 N.

3.3 Carrez solution I: dissolve 21.9 g of zinc acetate $Zn(CH_3COO)_2.2H_2O$ and 3 g of glacial acetic acid in water. Make up to 100 ml with water.

3.4 Carrez solution II: dissolve 10.6 g of potassium ferrocyanide $K_4[Fe(CN)_6].3H_2O$ in water. Make up to 100 ml with water.

3.5 Ethanol solution, 40% (v/v).

4. Apparatus

4.1 Erlenmeyer flask with standard ground-glass joint and with reflux condenser.

4.2 Polarimeter or saccharimeter.

5. Procedure

5.1 Preparation of the sample.

Crush the sample until it is fine enough for all of it to pass through a 0.5 mm round-meshed sieve.

5.2 Determination of the total optical rotation (P or S) (see item 7.1).

Weigh, to the nearest mg, 2.5 g of the crushed sample and place in a 100 ml graduated flask. Add 25 ml of hydrochloric acid (3.2), shake to obtain even distribution of the test sample and add a further 25 ml of hydrochloric acid (3.2). Immerse the flask in a boiling water bath shaking vigorously and steadily for the first three minutes to prevent the formation of agglomerates. The quantity of water in the water bath must be sufficient for the bath to remain at boiling point when the flask is introduced into it. The flask must not be taken out of the bath whilst being shaken. After exactly 15 minutes, remove from the bath, add 30 ml of cold water and cool immediately to 20°C.

Add 5 ml of Carrez solution I (3.3) and shake for 1 minute. Then add 5 ml of Carrez solution II (3.4) and shake again for 1 minute. Make up to volume with water, mix and filter. If the filtrate is not perfectly clear (which is rare), repeat the determination using a larger quantity of Carrez solutions I and II, for example 10 ml.

Measure the optical rotation of the solution in a 200 mm tube with the polarimeter or saccharimeter (4.2).

5.3 Determination of the optical rotation (P' or S') of substances soluble in 40% ethanol.

Weigh, to the nearest mg. 5 g of the sample, place in a 100 ml graduated flask and add about 80 ml of ethanol (3.5) (see observation 7.2). Leave the flask to stand for 1 hour at room temperature; during this time, shake vigorously on six occasions so that the test sample is thoroughly mixed with the ethanol. Make up to volume with ethanol (3.5), mix and filter.

Pipette 50 ml of the filtrate (=2.5 g of the sample) into an

Erlenmeyer flask, add 2.1 ml of hydrochloric acid (3.1) and shake vigorously. Fit a reflux condenser to the Erlenmeyer flask and immerse the latter in a boiling water bath. After exactly 15 minutes, remove the Erlenmeyer flask from the bath, transfer the contents to a 100 ml graduated flask, rinsing with a little cold water, and cool to 20°C.
Clarify using Carrez solutions I (3.3) and II (3.4), make up to volume with water, mix, filter and measure the optical rotation as indicated in the 2nd and 3rd paragraphs of 5.2.
6. Calculation of results
The starch content as a percentage of the sample is calculated as follows:
6.1 Measurement by polarimetry

D
where:
P=total optical rotation in degrees;P =optical rotation in degrees of substances soluble in 40% ethanol; (a)20
D=specific optical rotation of the pure starch. The conventionally accepted values for this factor are the following:
+185.9°: rice starch,
+185.7°: potato starch,
+184.6°: maize starch,
+182.7°: wheat starch,
+181.5°: barley starch,
+181.3°: oat starch,
+184.0° : other types of starch and starch mixtures in compound feedingstuffs.
6.2 Measurement by saccharimetry

D D
where:

S=total optical rotation in saccharimetric degrees;S' =optical rotation in saccharimetric degrees of substances soluble in 40% ethanol;N=weight in g of sucrose in 100 ml of water giving an optical rotation of 100 saccharimetric degrees in a 200 mm tube. The weight varies as follows according to the type of saccharimeter used:16.29 g for French saccharimeters,
26.00 g for German saccharimeters,
20.00 g for other saccharimeters;(a) 20°=specific optical rotation of the pure starch (see 6.1).D
7 Repeatability
The difference between the results of two parallel determinations carried out on the same sample must not exceed:
— 0.4% in absolute value, for starch contents of less than 40%.
— 1.0% relative to the higher results, for starch contents of 40% or more.
8. Observations
8.1 if the sample contains more than 6% of carbonates, calculated in terms of calcium carbonate, they must be destroyed by treatment with an exactly appropriate quantity of dilute sulphuric acid before determination of the total optical rotation.
8.2 In the case of products with a high lactose content, such as powdered whey or skimmed milk powder, proceed as follows after

adding 80 ml of ethanol (3.5). Fit a reflux condenser to the flask and immerse the latter in a water bath at 50°C for 30 minutes. Leave to cool and continue the analysis as indicated in 5.3.

3.2 DETERMINATION OF STARCH.

3.2.2 Pancreatic method

1. Purpose and scope

To determine the content of starch and of starch degradation products of high molecular weight in feedingstuffs containing beet cossettes, beet pulp. dried beet tops or leaves, potato pulp, dried yeasts, products rich in inulin (e.g. cossettes and meal of Jerusalem artichokes) and products containing greaves. The determination should only be carried out when microscopic examination shows that significant quantities of starch are present in the sample.

2. Principle

Sugars present in the sample are extracted with ethanol. The starch in the extracted residue is reduced to sugar with pancreatin. The sugars formed are hydrolysed with hydrochloric acid and the glucose formed is determined by the Luff-Schoorl method. The quantity of glucose thus obtained, multiplied by a constant factor, gives the starch content of the sample.

3. Reagents

3.1 Ethanol solution, 90% (v/v), neutral to phenolphthalein.

3.2 Pentane-1-ol (amyl alcohol).

3.3 Toluene.

3.4 Buffer solution: dissolve in water 9.078 g potassium dihydrogen phosphate KH_2PO_4 and 11.876 g disodium hydrogen phosphate. $Na_2HPO_4.2H_2O$. Make up to 1 litre with water.

3.5 Sodium chloride solution, 0.2N.

3.6 Carrez I solution : dissolve in water 21.9 g zinc acetate $Zn(CH_3COO)_2.2H_2O$ and 3 g glacial acetic acid. Make up to 100 ml with water.

3.7 Carrez II solution: dissolve in water 10.6 g potassium ferrocyanide $K_4[Fe(CN)_6].3H_2O$. Make up to 100 ml with water.

3.8 Hydrochloric acid, N.

3.9 Hydrochloric acid approximately 8N, d : 1.125.

3.10 Sodium hydroxide solution approximately 10N, d : 1.33.

3.11 Indicator: 0.1% (w/v) methyl orange solution.

3.12 Pancreatin in powder form, as per the directions in point 8. Keep in stoppered flasks, protected from light and moisture.

3.13 Luff-Schoorl reagent: pour the citric acid solution (3.13.2) into the Sodium carbonate solution (3.13.3) stirring carefully during the addition. Then add the copper sulphate solution (3.13.1) and make up to 1 litre with water. Leave to stand overnight and filter. Check the normality of the reagent thus obtained (Cu, 0.1 N; Na_2CO_3, 2N). The pH of the solution must be approximately 9.4.

3.13.1 Copper sulphate solution: dissolve 25 g copper sulphate $CuSo_4.5H_2O$ in 100 ml water.

3.13.2 Citric acid solution: dissolve 50 g citric acid $C_6H_8O_7H_2O$ in 50 ml water.

3.13.3 Sodium carbonate solution: dissolve 143.8 g anhydrous sodium carbonate in approximately 300 ml hot water. Leave to cool.

3.14 Granules of pumice stone purified by boiling in hydrochloric acid washing in water and drying.

3.15 Potassium iodide solution, 30% (w/v).

3.16 Sulphuric acid, approximately 6N, d : 1.18.
3.17 Sodium thiosulphate solution, 0.1N.
3.18 Starch solution: add a mixture of 5 g soluble starch in 30 ml water to 1 litre boiling water. Boil for 3 minutes, leave to cool. This solution should be freshly prepared.
4. Apparatus
4.1 Extractor (see diagram, fig. 1) consisting of:
4.1.1 A wide-necked 500 ml conical flask;
4.1.2 A reflux condenser fitted with a bung to the conical flask;
4.1.3 A sliding spindle in the centre of the reflux condenser, fitted with a hook at its lower end. A peg to hold the spindle firm;
4.1.4 A metal container for suspending on the spindle hook (4.1.3) and holding the filtration crucible (4.1.5);
4.1.5 A filtration crucible for rapid filtration; maximum size of pores: 90-150 microns (e.g. porosity 1), approximately 30 ml in capacity;
4.1.6 Filter papers, suitable in shape and size for the filtration crucible.
4.2 Incubator, set at 38°C.
4.3 200 ml graduated flasks with a standard ground glass joint and reflux condenser.
4.4 100 ml graduated flasks with a standard ground glass joint and reflux condenser.
5. Procedure
5.1 Preparation of the sample
Crush the sample so that the whole of it will pass through a 0.5 mm sieve. (ISO sieve of R 565 conforms).
5.2 Extraction
Weigh, to the nearest mg approximately 2 g of the sample and place in the filtration crucible (4.1.5), the bottom of which has first been covered with a filter paper (4.1.6) moistened with ethanol (3.1). Place in the conical flask (4.1.1) 55 ml ethanol (3.1) and a few granules of pumice stone (3.14). Place the filtration crucible in the metal container (4.1.4) and suspend the latter on the spindle hook (4.1.3). Place the reflux condenser over the conical flask and lower the spindle so that the bottom of the crucible just touches the surface of the ethanol. Peg the spindle firmly at this level. Bring the ethanol to boiling point and keep boiling for 3 hours. Then leave to cool and raise the spindle (4.1.3) to bring the crucible as high up as possible in the conical flask. Carefully unstopper the conical flask and allow 45 ml water to flow down along the sides of the flask. Replace the reflux condenser over the Erlenmeyer flask and keep the filtration crucible 10 cm above the surface of the liquid. Bring the liquid to boiling point and keep boiling for 3 hours. Then leave to cool, unstopper the conical flask and withdraw the crucible from the container.
5.3 Saccharification and hydrolysis
Place the crucible on a vacuum flask and dry under suction. Transfer the extraction residue to a mortar and grind finely. Using approximately 60 ml water, transfer the powder quantitatively to a 200 ml graduated flask with a standard ground glass joint and add a few drops of amyl alcohol (3.2). Connect a reflux condenser to the flask. Heat to boiling point and keep boiling for 1 hour. Then leave to cool and disconnect the reflux condenser. Add 25 ml buffer

solution (3.4), 250 mg pancreatin (3.12), 2.5 ml sodium chloride solution (3.5) and 10 drops toluene (3.3). Shake for 2 minutes, place the flask in the incubator (4.2) and keep there for 21 hours, shaking occasionally. Then leave to cool to room temperature. Add 5 ml Carrez I solution (3.6) and shake for 1 minute. Then add 5 ml Carrez II solution (3.7) and shake once again for 1 minute. Make up to volume with water, mix and filter. Using a pipette, take 50 ml filtrate and place in a 100 ml graduated flask (one may also work on 100 ml filtrate in a 200 ml graduated flask). Add a few drops of indicator (3.11) and acidify with hydrochloric acid 8 N (3.9) until the indicator turns red. Then add an extra 6.25 ml hydrochloric acid 8 N (3.9) (12.50 ml if working on 100 ml filtrate). Connect the reflux condenser to the flask, bring the solution to boiling point and keep boiling for 1 hour. Leave to cool, neutralise with the sodium hydroxide solution 10 N (3. 10) until the indicator turns yellow. Then acidify slightly by adding a little hydrochloric acid N (3.8), make up to volume with water and mix. Determine the glucose content according to the Luff-Schoorl method as shown in 5.4.

5.4 Titration according to Luff-Schoorl

Using a pipette, take 25 ml Luff-Schoorl reagent (3.13) and place in a conical flask; add 25 ml, accurately measured, of the solution obtained in 5.3; this should not contain more than 60 mg of glucose. Add two granules of pumice stone (3.14), heat, while shaking manually, over a medium flame and bring the liquid to boiling point in approximately 2 minutes. Immediately place the conical flask on a wire gauze fitted with an asbestos screen which has a hole approximately 6 cm in diameter. Under the wire gauze a flame has first been lit, and this is regulated in such a way that only the bottom of the conical flask is heated. Then connect a reflux condenser to the conical flask. As soon as this is done, boil for 10 minutes exactly. Cool immediately in cold water and after approximately 5 minutes, titrate as follows:

Add 10 ml potassium iodide solution (3.15) and, immediately afterwards, but with care, (because of the risk of extensive foaming) 25 ml sulphuric acid 6 N (3.16). Then titrate with the sodium thiosulphate solution 0.1 N (3.17) until a dull yellow colouring appears, add a few drops of starch indicator (3.18) and complete the titration.

Carry out the same titration on an accurately measured mixture of 25 ml Luff-Schoorl reagent (3.13) and 25 ml water, after adding 10 ml Potassium iodide solution (3.15) and 25 ml sulphuric acid 6 N (3.16), without bringing to the boil.

5.5 Blank Test

Carry out a blank test, applying the procedure described in 5.3 and 5.4, but without a sample.

6. Calculation of results

Using table 1 determine the quantity of glucose in mg corresponding to the difference between the results of the two titrations (expressed in ml of sodium thiosulphate 0. 1 N) and relating both to the sample analysis and the blank test.

The content of starch as a percentage of sample is given by the formula:

$$0.72\ (a - b)$$

where:

a = mg of glucose relating to the sample;
b = mg of glucose relating to the blank test (see item 7.2).
7. Observations
7.1 Where partially or totally dextrinated starch and lactose are simultaneously presented in the sample, the result may be high by 0.53.0%. In such cases, the actual starch content is obtained as follows:
(a) Determine the content of reducing sugars in the ethanolic extract obtained in 5.2, and express the result as a percentage of glucose;
(b) Determine the content of water-soluble reducing sugars in the sample, and express the result as a percentage of glucose;
(c) Deduct the result obtained in (a) from that obtained in (b) and multiply the difference by 0.9;
(d) Deduct the value obtained in (c) from the starch content obtained by applying the method and calculating as shown in 6.
7.2 The quantity of glucose in relation to the blank test is normally 0.25 mg. It may not be greater than 0.50 mg.
8. Directions relating to pancreatin
Physical aspect: yellowish-white, amorphous powder.
Glucose content: the quantity of glucose of the blank test (see 5.5) is normally 0.25 mg. A result greater than 0.50 mg indicates that pancreatin can no longer be used.
Check for iodine consumption: suspend 62.5 mg pancreatin in approximately 50 ml water heated to 2530°C. Add 1 ml iodine solution 0.1 N. Stir for 2 minutes. Titrate with a solution of sodium thiosulphate 0.1 N in the presence of starch indicator. Consumption of iodine solution by pancreatin must not exceed 0.5 ml.
Check for amylolytic activity: mix 100 ml starch solution (3.18), 5 ml buffer solution (3.4), 0.5 ml sodium chloride solution (3.5) and 62.5 mg pancreatin. Heat the mixture to 2530°C, stir for 2 minutes. Add 1 ml iodine solution 0.1 N. The blue colouration must have disappeared within 15 minutes exactly of the addition of the iodine solution.

Fig. 1.

TABLE 1.
Values for 25 ml Luff-Schoorl reagent (ml of Na2S2O3 0.1 N; 2 minutes heating, 10 minutes boiling)

Na2S2O3 O.1 NGlucose, fructose, invert sugars C6H12O6Lactose C12H22O11Maltose C12H22O11Na2S2O3 0.1 Nmlmgdifferencemgdifferencemgdifferenceml12.43.63.912.43.73.924.87.37.822.43.73.937.211.011.732.53.73.949.714.715.642.53.74.0512.218.419.652.53.73.9614.722.123.562.53.74.0717.225.827.572.63.74.0819.829.531.582.63.74.0922.433.235.592.63.84.01025.037.039.5102.63.84.01127.640.843.5112.73.84.01230.344.647.5122.73.84.11333.048.451.6132.73.84.11435.752.255,7142.83.84.11538.556.059.8152.83.94.11641.359.963.9162.93.94.11744.263.868.0172.93.94.21847.167.772.2182.94.04.31950.071.776.5193.04.04.42053.075.780.9203.04.14.52156.079.885.4213.14.14.62259.183.990.0223.14.14.62362.288.094.623
3.3 DETERMINATION OF SUGAR.
1. Purpose and scope
To determine the amount of reducing sugars and total sugars after inversion expressed as glucose or where appropriate as sucrose, converting by the factor 0.95. It is applicable to compound

feedingstuffs. Special procedures are provided for other feedingstuffs. Where necessary, lactose should be measured separately and taken into account when calculating the results.

2. Principle

The sugars are extracted in dilute ethanol; the solution is clarified with Carrez solutions I and II. After eliminating the ethanol, the quantities before and after inversion are determined by the Luff-Schoorl method.

3. Reagents

3.1 Ethanol solution, 40% (v/v), d : 0.948 at 20°C, neutralised to phenolphthalein.

3.2 Carrez solution I: dissolve in water 21.9 g of zinc acetate $Zn(CH_3COO)_2.2H_2O$ and 3 g of glacial acetic acid. Make up to 100 ml with water.

3.3 Carrez solution II: dissolve in water 10.6 g of potassium ferrocyanide $K_4[Fe(CN)_6].3H_2O$. Make up to 100 ml with water.

3.4 Methyl orange solution, 0.1% (w/v).

3.5 Hydrochloric acid, 4 N.

3.6 Hydrochloric acid, 0.1 N.

3.7 Sodium hydroxide solution, 0.1 N.

3.8 Luff-Schoorl reagent: stirring carefully, pour the citric acid solution (3.8.2) into the sodium carbonate solution (3.8.3). Add the copper sulphate solution (3.8.1) and make up to 1 litre with water. Leave to settle overnight and filter. Check the normality of the reagent thus obtained (Cu, 0.1 N; Na_2CO_3, 2N). The solution's pH should be approximately 9.4.

3.8.1 Copper sulphate solution: dissolve 25 g of copper sulphate, $CuSO_4.5H_2O$, free from iron, in 100 ml of water.

3.8.2 Citric acid solution: dissolve 50 g of citric acid, $C_6H_8O_7.H_2O$ in 50 ml of water.

3.8.3 Sodium carbonate solution: dissolve 143.8 g of anhydrous sodium carbonate in approximately 300 ml of warm water. Leave to cool.

3.9 Sodium thiosulphate solution 0.1 N.

3.10 Starch solution: add a mixture of 5 g of soluble starch in 30 ml of water to 1 litre of boiling water. Boil for 3 minutes, leave to cool and if necessary add 10 mg of mercuric iodide as a preservative.

3.11 Sulphuric acid, 6 N.

3.12 Potassium iodide solution, 30% (w/v).

3.13 Granulated pumice stone boiled in hydrochloric acid, washed in water and dried.

3.14 3-methylbutane-1-ol.

4. Apparatus

Shaker, approximately 35-40 rpm.

5. Procedure

5.1 Extraction of sample

Weigh, to the nearest mg approximately 2.5 g of the sample and place in a 250 ml volumetric flask. Add 200 ml of ethanol (3.1) and shake in the shaker for 1 hour. Add 5 ml of Carrez solution 1 (3.2) and stir for 1 minute. Add 5 ml of Carrez solution II (3.3) and again stir for 1 minute. Make up to volume with ethanol (3.1), mix and filter. Remove 200 ml of the filtrate and evaporate to approximately half volume in order to eliminate most of the ethanol. Transfer the evaporation residue quantitatively to a 200 ml volumetric flask using warm water, cool, bring up to volume with

water, mix and filter if necessary. This solution will be used to determine the amount of reducing sugars, and, after inversion, of total sugars.

5.2 Determination of reducing sugars

Using a pipette, remove not more than 25 ml of the solution containing less than 60 mg of reducing sugars expressed as glucose. If necessary, make up to 25 ml with water and determine the content of reducing sugars by the Luff-Schoorl method. The result is expressed as the percentage content of glucose in the sample.

5.3 Determination of total sugars after inversion

Using a pipette take 50 ml of the solution and transfer to a 100 ml volumetric flask. Add a few drops of methyl orange solution (3.4) then, carefully and stirring continuously, add hydrochloric acid 4 N (3.5) until the liquid turns a definite red. Add 15 ml of hydrochloric acid 0.1 N (3.6), immerse the flask in a fast boiling water bath and keep there for 30 minutes. Cool rapidly to approximately 20°C and add 15 ml of sodium hydroxide solution 0.1 N (3.7). Make up to 100 ml with water and mix. Remove not more than 25 ml containing less than 60 mg of reducing sugars expressed as glucose. If necessary, make up to 25 ml with water and determine the content of reducing sugars by the Luff-Schoorl method. The result is expressed as the percentage of glucose or, where appropriate, sucrose, by multiplying by the factor 0.95.

5.4 Titration by the Luff-Schoorl method

Using a pipette, take 25 ml of Luff-Schoorl reagent (3.8) and transfer to a 300 ml Erlenmeyer flask; add exactly 25 ml of the clarified sugar solution. Add 2 granules of pumice stone (3.13), heat, stirring by hand, over a free flame of medium height and bring the liquid to the boil in approximately 2 minutes. Place the Erlenmeyer immediately on an asbestos-coated wire gauze with a hole approximately 6 cm in diameter under which a flame has been lit. The flame shall be regulated in such a way that only the base of the Erlenmeyer is heated. Fit a reflux condenser to the Erlenmeyer flask. Boil for exactly 10 minutes. Cool immediately in cold water and after approximately 5 minutes titrate as follows:

Add 10 ml of Potassium iodide solution (3.12) and immediately afterwards (carefully, because of the risk of abundant foaming), add 25 ml of sulphuric acid 6 N (3.11). Titrate with sodium thiosulphate solution 0.1 N (3.9) until a dull yellow colour appears, add the starch indicator (3.10) and complete titration.

Carry out the same titration on an accurately measured mixture of 25 ml of Luff-Schoorl reagent (3.8) and 25 ml of water, after adding 10 ml of potassium iodide solution (3.12) and 25 ml of sulphuric acid 6 N (3.11) without boiling.

6. Calculation of results

Using table 1 establish the amount of glucose in mg which corresponds to the difference between the values of two titrations, expressed in mg of sodium thiosulphate 0.1 N.

Express the result as a percentage of the sample.

7. Special procedures

7.1 In the case of feedingstuffs which are rich in molasses and other feedingstuffs which are not particularly homogeneous, weigh out 20 g and place with 500 ml of water in a 1 litre volumetric flask. Mix for 1 hour in the shaker. Clarify using Carrez I (3.2) and II (3.3) reagents as described under 5.1, this time however

using four times the quantities of each reagent. Bring up to volume with 80% ethanol (v/v).
Mix and filter. Eliminate the ethanol as described under 5.1. If there is no dextrinised starch, bring up to volume with water.
7.2 In the case of molasses and straight feedingstuffs which are rich in sugar and almost starch-free (carobs, dried beetroot, cossettes, etc.) weigh out 5 g place in a 250 ml volumetric flask, add 200 ml of water and mix in the shaker for 1 hour, or more if necessary. Clarify using Carrez I (3.2) and II (3.3) reagents as described under 5.1. Bring up to volume with cold water, mix and filter. In order to determine the amount of total sugars, continue as described under 5.3.
8. Observations
8.1 In order to prevent foaming it is advisable to add (irrespective of the volume) approximately 1 ml of 3-methylbutane-1-ol (3.14) before boiling with Luff-Schoorl reagent.
8.2 The difference between the content of total sugars after inversion expressed as glucose, and the content of reducing sugars, expressed as glucose, multiplied by 0.95, gives the percentage content of sucrose.
8.3 In order to determine the content of reducing sugars, excluding lactose, two methods may be adopted:
8.3.1 For an approximate calculation, multiply by 0.675 the lactose content established by a different method of analysis and subtract the result obtained from the content of reducing sugars.
8.3.2 For an accurate calculation of reducing sugars, excluding lactose, the same sample must be used for the two final determinations. One of the analyses is carried out on part of the solution obtained under 5.1, the other on part of the solution obtained during the determination of lactose by the method laid down for that purpose (after fermenting the other types of sugar and clarifying).
In both cases the amount of sugar present is determined by the Luff-Schoorl method and calculated in mg of glucose. One of the values is subtracted from the other and the difference is expressed as a percentage of the sample.
TABLE 1.
Values for 25 ml Luff-Schoorl reagent (ml of $Na_2S_2O_3$ 0.1 N; 2 minutes heating, 10 minutes boiling)

$Na_2S_2O_3$
O.1 NGlucose, fructose, invert sugars
$C_6H_{12}O_6$Lactose
$C_{12}H_{22}O_{11}$Maltose
$C_{12}H_{22}O_{11}Na_2S_2O_3$
O.1
Nmlmgdifferencemgdifferencemgdifferenceml12.43.63.912.43.73.924.87.37.822.43.73.937.211.011.732.53.73.949.714.715.642.53.74.0512.218.419.652.53.73.9614.722.123.562.53.74.0717.225.827.572.63.74.0819.829.531.582.63.74.0922.433.235.592.63.84.01025.037.039.5102.63.84.01127.640.843.5112.73.84.01230.344.647.5122.73.84.11333.048.451.6132.73.84.11435.752.255.7142.83.84.11538.556.059.8152.83.94.11641.359.963.9162.93.94.11744.263.868.0172.93.94.21847.167.772.2182.94.04.31950.071.776.5193.04.04.42053.075.780.9203.04.14.52156.079.885.4213.14.14.62259.183.990.0223.14.14.62362.288.094.623
Example
The two volumes taken correspond, for each determination, to a

sample of 250 mg.
In the first case 17 ml of sodium thiosulphate solution 0.1 N corresponding to 44.2 mg of glucose is consumed; in the second, 11 ml corresponding to 27.6 mg of glucose.
The difference is 16.6 mg of glucose.
The content of reducing sugars (excluding lactose), calculated as glucose, is therefore:

3.4 DETERMINATION OF LACTOSE.
1. Purpose and scope
To determine the level of lactose in feedingstuffs containing more than 0.5% of lactose.
2. Principle
The sugars are dissolved in water. The solution is subjected to fermentation by the yeast Saccharomyces cerevisiae which leaves the lactose intact. After clarification and filtration the lactose content of the filtrate is determined by the Luff-Schoorl method.
3. Reagents
3.1 Suspension of Saccharomyces cerevisiae: suspend 25 g of fresh yeast in 100 ml of water. The suspension will keep for a maximum period of 1 week in a refrigerator.
3.2 Carrez solution I: dissolve in water 21.9 g of zinc acetate Zn(CH-3COO)2.2H2O and 3 g of glacial acetic acid. Make up to 100 ml with water.
3.3 Carrez solution II: dissolve in water 10.6 g of potassium ferrocyanide K4[Fe(CN)6].3H2O. Make up to 100ml with water.
3.4 Luff-Schoorl reagent: stirring carefully, pour the citric acid solution (3.4.2) into the sodium carbonate solution (3.4.3). Add the copper sulphate solution (3.4.1) and make up to 1 litre with water. Leave to settle over night and filter. Check the normality of the reagent thus obtained (Cu 01 N; Na2CO32N). The solution's pH should be approximately 9.4.
3.4.1 Copper sulphate solution: dissolve 25 g of copper sulphate CuSO4.5H2O, free from iron, in 100 ml of water.
3.4.2 Citric acid solution: dissolve 50 g of citric acid C6H8O7.H2O in 50 ml of water.
3.4.3 Sodium carbonate solution: dissolve 143.8 g of anhydrous sodium carbonate in approximately 300 ml of warm water. Leave to cool.
3.5 Granulated pumice stone boiled in hydrochloric acid, washed in water and dried.
3.6 Potassium iodide solution 30% (w/v).
3.7 Sulphuric acid, 6 N.
3.8 Solution of sodium thiosulphate, 0.1 N.
Starch solution: add a mixture of 5 g of soluble starch in 30 ml of water to 1 litre of boiling water. Boil for 3 minutes, leave to cool, and if necessary add 10 mg mercuric iodide as a preservative.
4. Apparatus
Water bath with thermostat set at 38-40°C.
5. Procedure
Weigh, to the nearest mg approximately 1 g of the sample and place this portion of the sample in a 100 ml volumetric flask. Add 25-30 ml of water. Place the flask in a boiling water bath for 30 minutes and then cool to approximately 35%C. Add 5 ml of yeast suspension (3.1) and mix. Leave the flask to stand for 2 hours in

a water bath, at a temperature of 38-40°C. Cool to approximately 20°C.
Add 2.5 ml of Carrez solution I (3.2) and stir for 30 seconds, then add 2.5 ml of Carrez solution II (3.3) and again stir for 30 seconds. Make up to 100 ml with water, mix and filter. Using a pipette, remove an amount of filtrate which does not exceed 25 ml and which preferably contains from 40-80 mg of lactose and transfer it to a 300 ml Erlenmeyer flask. If necessary, make up to 25 ml with water.
Carry out a blank test in the same way with 5 ml of yeast suspension (3.1).
Determine the lactose content according to Luff-Schoorl, as follows: add exactly 25 ml of Luff-Schoorl reagent (3.4) and two granules of pumice stone (3.5). Stir by hand while heating over a free flame of medium height and bring the liquid to the boil in approximately 2 minutes. Place the Erlenmeyer flask immediately on an asbestos-coated wire gauze with a hole approximately 6 cm in diameter under which a flame has been lit. The flame shall be regulated in such a way that only the base of the Erlenmeyer flask is heated. Fit a reflux condenser to the Erlenmeyer flask. Boil for exactly 10 minutes. Cool immediately in cold water and after approximately 5 minutes titrate as follows:
Add 10 ml of potassium iodide solution (3.6) and immediately afterwards (carefully, because of the risk of abundant foaming) add 25 ml of sulphuric acid 6 N (3.7). Titrate with sodium thiosulphate solution 0.1 N (3.8) until a dull yellow colour appears, add the starch indicator (3.9) and complete titration.
Carry out the same titration on an accurately measured mixture of 25 ml of Luff-Schoorl reagent (3.4) and 25 ml of water, after adding 10 ml. of potassium iodide solution (3.6) and 25 ml of sulphuric acid 6N (3.7) without boiling.
6. Calculation of results
Using the attached table, establish the amount of lactose in mg which corresponds to the difference between the results of the two titrations, expressed in ml of sodium thiosulphate 0.1 N.
Express the result as a percentage of anhydrous lactose in the sample.
TABLE 1.
Values for 25 ml Luff-Schoorl reagent (ml of $Na_2S_2O_3$ 0.1 N; 2 minutes heating, 10 minutes boiling)

$Na_2S_2O_3$
O.1 NGlucose, fructose, invert sugars $C_6H_{12}O_6$Lactose $C_{12}H_{22}O_{11}$Maltose $C_{12}H_{22}O_{11}$$Na_2S_2O_3$
O.1Nmlmgdifferencemgdifferencemgdifferenceml12.43.63.912.43.73.924.87.37.822.43.73.937.211.011.732.53.73.949.714.715.642.53.74.0512.218.419.652.53.73.9614.722.123.562.53.74.0717.225.827.572.63.74.0819.829.531.582.63.74.0922.433.235.592.63.84.01025.037.039.5102.63.84.01127.640.843.5112.73.84.01230.344.647.5122.73.84.11333.048.451.6132.73.84.11435.752.255.7142.83.84.11538.556.059.8152.83.94.11641.359.963.9162.93.94.11744.263.868.0172.93.94.21847.167.772.2182.94.04.31950.071.776.5193.04.04.42053.075.780.9203.04.14.52156.079.885.4213.14.14.62259.183.990.0223.14.14.62362.288.094.623
7. Observation
For products containing more than 40% of fermentable sugar, use more than 5 ml of yeast suspension (3.1).

4. OILS AND FATS.

4.1 DETERMINATION OF CRUDE OILS AND FATS.

1. Purpose and Scope

To determine the content of crude oils and fats in feeding stuffs.

It does not cover the analysis of the oil seeds and oleaginous fruit defined in Council Regulation 136/66/EEC1 of 22 September 1966. (See method 4.2)

1 OJ No. 127, 30 September 1966, P.3025/66.

Depending on the nature of the feedingstuff, either of the two methods described must be used.

1.1 Method A

Applicable to straight feedingstuffs of plant origin, with the exception of those which are known to contain oils and fats which cannot be totally extracted with light petroleum without prior hydrolysis. Among these are glutens, yeasts, soya and potato proteins. This method is also applicable to compound feedingstuffs, with the exception of those which contain milk powder or from which oils and fats cannot be totally extracted with light petroleum without prior hydrolysis.

1.2 Method B

Applicable to straight feedingstuffs of animal origin as well as to feedingstuffs mentioned under point 1.1 as being exceptions for method A.

2. Principle

2.1 Method A

The sample is extracted with light petroleum. The solvent is distilled off and the residue dried and weighed.

2.1 Method B

The sample is treated under heating with hydrochloric acid. The mixture is cooled and filtered. The residue is washed and dried and submitted to the determination according to method A.

3. Reagents

3.1 Light petroleum, boiling range: 40 to 60°C. The bromine value must be less than 1 and the residue of evaporation less than 2 mg/100 ml.

3.2 Sodium sulphate, anhydrous.

3.3 Hydrochloric acid, 3 N.

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

4. Apparatus

4.1 Extraction apparatus. If fitted with a siphon (Soxhlet apparatus), the reflux rate should be such as to produce about 10 cycles per hour; if of the non-siphoning type, the reflux rate should be about 10 ml per minute.

4.2 Extraction thimbles, free of matter soluble in light petroleum and having a porosity consistent with the requirements of point 4.1.

4.3 Drying oven, either a vacuum oven set at 75±3°C or an air-oven set at 100±3°C.

5. Procedure

5.1 Method A (see point 8.1)

Weigh to the nearest mg, approximately 5 g of the sample and transfer it to an extraction thimble (4.2) and cover with a fat-free wad of cotton wool.

Place the thimble in an extractor (4.1) and extract for six hours with light petroleum (3.1). Collect the light petroleum extract in a dry, weighed flask containing fragments of pumice stone1.

Distill off the solvent. Dry the evaporation residue maintaining the flask for one and a half hours in the drying oven (4.3). Leave to cool in a dessiccator and weigh. Dry again for 30 minutes to ensure that the weight of the oils and fats remains constant (loss in weight between two successive weighings must be less than 1 mg).

5.2 Method B

Weigh to the nearest mg, approximately 2.5 g of sample (see point 8.2) and place it in a conical flask. Add 100 ml of hydrochloric acid 3 N (3.3), some fragments of pumice stone and fit 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 a neutral filtrate is obtained. Check that the filtrate does not contain any oil or fats. Their presence indicates that the sample must be extracted with light petroleum, using method A, 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 100±3°C.

Place the double filter paper containing the dry residue in an extraction thimble (4.2) and cover with a fat-free wad of cotton wool. Place the thimble in an extractor (4.1) and proceed as indicated in the second and third paragraphs of point 5.1.

6. Expression of result

Express the weight of the residue as a percentage of the sample.

7. Repeatability

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

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

0.2% in absolute value, for contents of crude oils and fats lower than 5%,

4.0% related to the highest result for contents of 5 to 10%,

0.4% in absolute value, for contents above 10%.

8. Observations

8.1 For products with a high content of oils and fats, 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 light petroleum (3.1) as indicated in point 5.1. Make up the extract obtained to 500 ml with light petroleum (3.1) and homogenize. Take 50 ml of the solution and place in a small, dry, weighed flask containing fragments of pumice stone 1. Distill off the solvent, dry and proceed as indicated in the last paragraph of point 5.1.

Eliminate the solvent from the extraction residue left in the thimble, crush the residue to a fineness of 1 mm, return it to the extraction thimble (do not add sodium sulphate) and proceed as indicated in the second and third paragraphs of point 5.1.

Calculate the content of oils and fats as a percentage of the sample by using the following formula:

$(10a + b)x5$

where:
a=mass in grams of the residue after the first extraction (aliquot part of the extract),
b=mass in grams of the residue after the second extraction.
8.2 For products low in oils and fats the test sample may be increased to 5 g.
4.2 DETERMINATION OF OIL CONTENT IN OLEAGINOUS SEEDS.
1. Purpose and Scope
To determine the oil content in oleaginous seeds.
2. Principle
The sample is extracted with n-hexane. The solvent is removed by distillation and the residue dried and weighed.
3. Reagents
3.1 n-Hexane.
3.2 Sand, acid washed.
3.3 Hydrochloric acid: d=1.18.
4. Apparatus
4.1 Soxhlet-type extractor or equivalent apparatus.
4.2 Electric oven with temperature regulation.
1Where the oil or fat has to undergo subsequent quality tests, replace the fragments of pumice stone by glass beads.
4.3 Metal vessel, flat-bottomed, diameter about 100 mm, height about 40 mm.
4.4 Porous vessel of ceramic material, cylindrical, internal diameter 68 mm, external diameter 80 mm, height 85 mm, thickness of walls and base 6mm.
4.5 Fumigation oven, with temperature control.
5. Procedure
5.1 Preparation.
5.1.1 Copra.
Grate the product with a hand grater or preferably with the mechanical grater which can deal with the whole sample for analysis. Hand operation does not allow the whole sample for analysis to be grated. Endeavour to obtain a sub-sample which is as representative as possible. The thickness and the colour of the different pieces should be taken into account.
The length of the grated particles may exceed 2 mm, but should not be greater than 5 mm.
Mix the particles carefully and carry out the determination without delay.
5.1.2 Medium-sized seeds (e.g. sunflower, groundnut, soya) with the exception of cottonseed with adherent linters.
Crush the sample for analysis, in the previously well cleaned mechanical mill until particles are obtained with a major dimension not greater than 2 mm. Reject the first grindings (about 1/20 of the sample) and collect the rest. Mix carefully and carry out the determination without delay.
5.1.3 Cottonseed with adherent linters weigh into the tared metal vessel (4.3) and to the nearest 10 mg, 60 g of sample. Put the vessel and seed in the oven previously heated to 130°C and leave to dry for two hours at 130E2°C, then remove the vessel from the oven and allow to cool in air for about thirty minutes. Transfer the dried seed to the porous ceramic vessel (4.4), the inside walls and base of which have been previously moistened with 1.5 ml of concentrated hydrochloric acid (3.3) by means of the pipette taking

care that the acid is completely absorbed without forming adherent drops. Close the vessel with the watch glass and put in the fumigation oven (4.5). Heat so as to reach 115°C in thirty minutes; do not heat beyond this temperature and maintain it for another thirty minutes.

Take the vessel out of the oven, allow to cool for one hour in air, reweigh the treated seed to the nearest 10 mg, then grind the seed in the mechanical mill and continue as described in clause 5.1.2.

5.1.4 Small seeds (e.g. linseed colza etc.) analyse without grinding.

5.2 Weighing

5.2.1 Seeds coming under, 5.1.1, 5.1.2 and 5.1.3. Weigh to the nearest 10 mg, 10 g of sample, place in an extraction thimble and plug with a wad of cotton wool.

5.2.2 Seeds coming under 5.1.4

Weigh to the nearest 10 mg, 10 g of sample and grind in a microgrinder, taking care not to leave any seeds intact. Transfer, without loss, the ground seeds to the extraction thimble, using a spatula. Wipe the bowl of the micro-grinder and the spatula, with a wad of cotton wool soaked with solvent (3.1) and plug the thimble with this wad.

5.3 Determination.

If the moisture content is greater then 10% place the thimble and sample in an oven set at a temperature not exceeding 80°C and leave there for some time, until the moisture content is reduced to less than 10%.

Place the thimble in the extraction apparatus (4.1) and extract for four hours with n-hexane (3.1). Regulate the heating so that the reflux rate is at least three drops per second. Collect the extract in a dry weighed flask marked A.

After extracting for four hours, allow to cool. Remove the thimble from the extractor and place it in a current of air in order to remove the greater part of the solvent. Empty the thimble into the mortar, add about 10 g of sand (3.2) and triturate as finely as possible. Replace the mixture in the thimble and the latter in the extractor, and continue the extraction for two hours, using the same flask.

Leave to cool, remove the thimble again, eliminate the solvent and repeat the trituration as above (without further addition of sand). Carry out a third extraction for two hours, collecting the product this time in a dried weighed flask marked B.

Remove the greater part of the solvent, by distillation and a boiling water bath, from flask A and B. Remove the last traces of solvent by heating the flasks for twenty minutes at 103±2°C. Assist the elimination either by blowing in air at intervals or by using reduced pressure. Leave the flask to cool in a desiccator, for at least one hour, and weigh to the nearest 1 mg. Heat again for ten minutes under the same conditions, cool and weigh. The difference between these two weighings should not exceed 10 mg. If it does so, heat again for ten minutes, until the difference in mass is not greater than 10 mg. Keep a note of the final mass of flask A.

If the mass of oil in flask B does not exceed 10 mg the operation is complete. If it does so, carry out a fresh extraction for two hours, using flask B, until the mass of oil from the last

extraction is not more than 0.010 g.
6. Calculation of results
6.1 For all seeds other than cottonseed with adhering linters.
The oil content, as a percentage of the sample, is calculated by using the following formula.

M1 is the mass in grams of oil in flasks A & B.
Mo is the mass in grams of the test sample.
6.2 Cottonseed with adhering linters.
The oil content, as a percentage of the sample, is calculated by using the following formula.

M1 and Mo have the same meaning as par 6.1.
M2 = mass in grams of test portion before pre treatment (5.1.3).
M3 = mass in grams of test portion (5.1.3) after pre treatment.
7. Repeatability
The difference between the results of two parallel determinations carried out of the same sample should not exceed 0.4% in absolute value.
4.3 DETERMINATION OF ACID INDEX.
4.3.1 Indicator method
1. Purpose and scope
To determine the acid index in animal and vegetable fats and oils. Highly coloured oils and fats may interfere in the determination.
2. Principle
The acid index is expressed as the number of mg of potassium hydroxide required to neutralise the free fatty acids in 1 g of the sample.
3. Reagents
3.1 Neutralise, just before use, a 1 : 1 mixture of 95% ethanol (V/V) and diethyl ether, with Potassium hydroxide solution (3.2) using phenolphthalein (3.3) as indicator.
3.2 Ethanolic potassium hydroxide. 0.1 N or 0.5 N. Prepare at least five days prior to use and standardise immediately before use.
3.3 Phenolphthalein solution; dissolve 1 g in 100 ml of 95% (V/V) ethanol.
4. Procedure
Weigh to the nearest mg, a quantity of sample indicated in the table and place in a conical flask.

Expected acid indexWeight of test portion in g <1201 — 4104 — 15 2.515 — 75 0.5>750.1
Dissolve it in about 150 ml of solvent mixture (3.1) and titrate with potassium hydroxide (3.2) using phenolphthalein (3.3) as indicator. If the quantity of 0.1 N Potassium hydroxide solution required exceeds 20 ml a 0.5 N solution should be used.
5. Calculation of results
The acid index (A.I.) is calculated using the formula:

where V is the number of ml of the standardized potassium hydroxide solution (3.2) used,
T is the exact normality of the standardized potassium hydroxide solution (3.2),
m is the weight in g, of the test sample.
4.3 DETERMINATION OF ACID INDEX.
4.3.2 Potentiometric method

1. Purpose and scope
To determine the acid index in animal and vegitable oils and fats, which are highly coloured.
2. Principle
The acid index is expressed as the number of mg of potassium hydroxide required to neutralise the free fatty acids in 1 g of sample.
3. Reagents
3.1 Propan-2-ol.
3.2 Methylisobutylketone, analytical reagent quality, neutralized immediately before use with potassium hydroxide solution (3.4) to a faint pink colour with phenolphthalein indicator.
3.3 Benzoic acid.
3.4 Potassium hydroxide, 0.1 N solution in propan-2-ol (3.1). Dissolve 7 g of potassium hydroxide pellets in 1000 ml of propan-2-ol (3.1).
3.5 Potassium hydroxide, 0.5 N solution in propan-2-ol. Dissolve 35 g of potassium hydroxide pellets in 1000 ml of propan-2-ol (3.1).
Standardise the solutions as follows: weigh accurately to within 0.0002 g about 0.15 g (for the 0.1 N solution) or 0.75 g (for the 0.5 N solution) of benzoic acid (3.3). Transfer into a beaker and dissolve in 50 ml of methylisobutylketone (3.2).
Insert the electrodes of the pH meter (4.1), start the stirrer (4.2) and titrate with the potassium hydroxide solution (3.4 or 3.5) to the equivalence point (see 7 Observation).

where ao is the number of ml of the potassium hydroxide solution (5.4.4 or 5.4.5) used,
mo is the weight, in g, of benzoic acid taken.
4. Apparatus
4.1 pH meter.
4.2 Magnetic stirrer.
5. Procedure
Weigh to the nearest mg and directly into a beaker 5-10 g of sample. Dissolve it in 50 ml of methylisobutylketone (3.2). Insert the electrodes of the pH meter (4.1) and start the stirrer (4.2). Titrate with the potassium hydroxide solution (3.4 or 3.5) 0.1 or 0.5 N. (according to the acidity of the sample) to the equivalence point (see 7 Observation).
6. Calculation of Results
The acid index (A.I.) is given by the formula:

where V is the number of ml of the standardised isopropanolic potassium hydroxide solution (3.4 or 3.5) used,
T is the exact normality of the standardised isopropanolic potassium hydroxide solution (3.4 or 3.5) used,
m is the weight, in g, of the test portion.
7. Observation
The equivalence point usually corresponds approximately to the reading 10 on the pH scale, and can be determined graphically by observing the inflexion point on the neutralization curve. Alternatively, it can be calculated as the figure for which the first differential of the variation of pH as a function of the amount of isopropanolic potassium hydroxide solution added reaches a maximum, or the value of which the second differential becomes zero.

N.B. It is not possible to determine the inflexion piont in the case of crude cottonseed oils rich in gossypol.
In this case the inflexion point may be taken arbitrarily as the pH corresponding to the equivalence point when oleic acid is neutralized by potassium hydroxide in the same solvent as that used for the titration.
Dissolve about 0.282 g of oleic acid in 50 ml of methylisobutylketone (3.2). Construct the curve for neutralization of the oleic acid by the potassium hydroxide solution (3.4 or 3.5) to be used. Read off on the curve the pH of the inflexion point (representing theoretically the addition of 10 ml of a 0.1 N solution of potassium hydroxide).
Apply this figure to the neutralisation curve of the cottonseed oil to deduce the amount of potassium hydroxide solution required to "neutralise" the cottonseed oil.

4.4 DETERMINATION OF MATTER INSOLUBLE IN LIGHT PETROLEUM.

1. Purpose and Scope

To determine the matter insoluble in light petroleum in oils and fats.

2. Principle

The sample is treated with light petroleum and the insoluble residue is determined after filtration and drying. To ensure even distribution of the impurities, the sample may, if necessary, be melted and then shaken or stirred until cool.

3. Reagents

3.1 Light petroleum; boiling range 40-60°C.

3.2 Diatomaceous earth purified.

4. Apparatus

4.1 Glass filter crucible, 10-16 mm pore diameter, 40 mm ID.

5. Procedure

Weigh to the nearest 0.01 g (for samples with a high impurity content, the weight of the sample must be lower) 5 g of the sample into the glass beaker; add 100 ml of pre-heated light petroleum (3.1) (to be heated under a reflux condenser) and stir with the glass rod until the fat has dissolved. The beaker can then be covered with the watchglass and kept at about 30°C for 30 min to ensure that the sample is completely dissolved. The diatomaceous earth filter is prepared as follows: stir 2 g diatomaceous earth in a glass beaker with about 30 ml light petroleum, transfer to a filter crucible (4.1) and apply a vacuum in order to produce a filter bed. After all the light petroleum has been suctioned off, dry the glass filter crucible in a drying oven at 130°C for 10 minutes; then place the crucible in a desiccator to cool, and finally weigh to the nearest 0.1 mg (b).
While applying a vacuum, filter the contents of the glass beaker through the glass filter crucible (4.1) prepared in accordance with the previous paragraph, taking care that the fat is fully dissolved in the pre-heated light petroleum, and wash the residue with about 100 ml of warmed light petroleum. After all the light petroleum has been suctioned off, dry the crucible for 10 minutes in the drying oven at 130°C ± 2°C, place it in the desiccator to cool and then weigh it (a). Repeat the drying and weighing until constant weight is achieved (deviation <y5 mg).

6. Calculation of results

The quantity of matter insoluble in light petroleum (I) in the

sample is expressed as a percentage by means of the following formula:

where a = weight of glass filter crucible with silica gel filter and residue in grams.
b = weight of glass filter crucible with diatomaceous earth filter in grams. E= weight of sample in grams.
7. Repeatability
4% relative to the higher result.
5. PROTEINS.
5.1 DETERMINATION OF CRUDE PROTEIN.
1. Purpose and scope
To determine the crude protein content of feedingstuffs on the basis of the nitrogen content, determined according to the Kjeldahl method.
2. Principle
The sample is digested by mineral acid. The acid solution is alkalized with a sodium hydroxide solution. The ammonia released is removed by distillation and collected in a measured quantity of sulphuric acid, the excess of which is titrated with a solution of sodium hydroxide.
3. Reagents
3.1 Potassium sulphate.
3.2 Catalyst: cupric oxide CuO or crystallized cupric sulphate CuSO4.5H2O or mercury or mercuric oxide HgO.
3.3 Granulated zinc.
3.4 Sulphuric acid, d : 1.84.
3.5 Sulphuric acid, 0.1 N.
3.6 Sulphuric acid, 0.5 N.
3.7 Methyl red indicator: dissolve 300 mg of methyl red in 100 ml of 95-96% (v/v) ethanol.
3.8 Sodium hydroxide solution, 40% (w/v).
3.9 Sodium hydroxide solution, 0.1 N.
3.10 Sodium hydroxide solution, 0.25 N.
3.11 Sodium sulphide, saturated solution.
3.12 Sodium thiosulphate solution, 8% (w/v) Na2S2O3.5H2O.
3.13 Granulated pumice stone, washed in hydrochloric acid and ashed.
4. Apparatus
Apparatus for digestion by combustion and for distillation by the Kjeldahl method (see item 7.1).
5. Procedure
5.1 Digestion
Weigh, to the nearest mg. approximately 1 g of sample and place in the flask of the digestion apparatus. Add 10 g of potassium sulphate (3.1), and appropriate quantity of catalyst (3.2) (0.3-0.4 g of cupric oxide or 0.9-1.2 g of cupric sulphate or a drop of mercury or 0.6-0.7 g of mercuric oxide), 25 ml of sulphuric acid (3.4) and a few granules of pumice stone (3. 13). Mix. Heat the flask moderately at first, shaking from time to time, until the mass has carbonized and the foam has disappeared; then heat more intensively until the liquid is boiling steadily. Prevent the sides from becoming overheated and organic particles from sticking to them. When the solution becomes clear and colourless (or light green if a copper-based catalyst is used), continue to boil for another hour, then leave to cool.
5.2 Distillation

Carefully add 250-350 ml of water, stirring all the while to dissolve the sulphates completely; leave to cool. Add a few granules of zinc (3.3).

Place in the collecting flask of the distillation apparatus and exactly measured quantity of 25 ml of sulphuric acid 0.1 N (3.5) or 0.5 N (3.6) depending on the presumed nitrogen content (see item 7.2), and add a few drops of methyl red indicator (3.7).

Connect the distillation flask to the condenser of the distillation apparatus and immerse the end of the condenser in the liquid contained in the collecting flask to a depth of at least 1 cm (see item 7.3). Slowly pour 100 ml of 40% sodium hydroxide solution (3.8) into the distillation flask through the dropping funnel. If a mercury-based catalyst has been used, also add either 10 ml of sodium sulphide solution (3.11), or 25 ml of sodium thiosulphate solution (3.12).

Heat the flask in such a way that approximately 150 ml of liquid is distilled in 30 minutes. At the end of this time, check the pH of the resulting distillate with litmus paper. If the reaction is alkaline, continue distillation. Discontinue when the distillate becomes neutral to litmus paper. During distillation keep the colouration under observation and shake the contents of the collecting flask from time to time. If the liquid turns yellow, immediately add an exactly measured volume of sulphuric acid 0.1 N (3.5) or 0.5 N (3.6).

5.3 Titration

In the collecting flask titrate the excess sulphuric acid with sodium hydroxide solution 0.1 N (3.9) or 0.25 N (3.10), depending on the normality of the sulphuric acid used, until the colour turns pale yellow.

5.4 Verification of the method

To establish whether the reagents are free from nitrogen, carry out a blank test (distillation and titration) omitting the sample to be analysed. To check that the apparatus is working properly and that the correct application of the method is used carry out the analysis (digestion, distillation and titration) on 1.5-2.0 g of acetanilide (m.p. 114°C : %N : 10.36) in the presence of 1 g of nitrogen-free sucrose; 1 g of acetanilide corresponds to 14.80 ml of sulphuric acid 0.5 N.

6. Calculation of results

Determine the volume of sulphuric acid neutralised. 1 ml of sulphuric acid 0.1 N corresponds to 1.4 mg of nitrogen. Multiply the quantity of nitrogen by the factor 6.25. Express the result as a percentage of the sample.

7. Repeatability

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

— 0.2%, in absolute value, for crude protein contents of less than 20% ;

— 1.0%, relative to the higher result, for contents of not less than 20% and not more than 40%;

— 0.4%, in absolute value, for contents of more than 40%.

8. Observations

8.1 Certain apparatus requiring transference between digestion and distilation may be used. If such apparatus is used, the transfer must be carried out without loss.

8.2 For products with a low nitrogen content, the volume of sulphuric acid 0.I N to be placed in the collecting flask may be reduced, if necessary, to 10 or 15 ml and made up to 25 ml with water.
8.3 If the flask of the distillation apparatus is not fitted with a dropping funnel, add the sodium hydroxide immediately before connecting the flask to the condenser, pouring the liquid slowly down the sides of the condenser so that it does not mix with the acid solution.

5.2 DETERMINATION OF CRUDE PROTEIN DISSOLVED BY PEPSIN AND HYDROCHLORIC ACID.

1. Purpose and scope

To determine the fraction of crude protein dissolved by pepsin and hydrochloric acid under defined conditions. It is applicable to all feedingstuffs.

2. Principle

The sample is heated for 48 hours at 40°C in a solution of pepsin hydrochloride. The suspension is filtered and the nitrogen content of the filtrate determined according to the method for the determination of crude protein.

3. Reagents

3.1 Hydrochloric acid, d : 1.125.
3.2 Hydrochloric acid, 0.075 N.
3.3 2.0 U/mg pepsin; pepsin activity is defined in the method for the Estimation of Pepsin Activity specified in these Regulations, and must be established according to that method.
3.4 About 0.2% (w/v) freshly prepared solution of pepsin in hydrochloric acid (3.2); activity: 400 U/litre.
3.5 Anti-foaming emulsion (e.g. silicone).
3.6 All reagents listed under 3 in the method for the determination of crude protein. (Method 5.1).

4. Apparatus

4.1 Water bath or incubator, set at 40°C ± 1°C.
4.2 Kjeldahl digestion and distillation apparatus.

5. Procedure

5.1 Preparation of solution (see item 7.2).
Weigh, to the nearest mg, approximately 2.g of the sample and place in a 500 ml graduated flask. Add 450 ml of pepsin hydrochloride solution (3.4) previously heated to 40°C and shake to prevent the formation of agglomerates. Check that the pH of the suspension is less than 1.7. Place the flask in the water bath or incubator (4.1) and leave there for 48 hours. Shake after 8, 24 and 32 hours. After 48 hours, add 15 ml of hydrochloric acid (3.1), cool to 20°C, make up to volume with water and filter.
5.2 Digestion
Take 250 ml of the filtrate and place in the flask of the distillation apparatus (4.2). Add the reagents necessary for digestion indicated in the second sentence of 5.1 of the method for the determination of crude protein. Mix and bring to the boil. If any foam should form, add a few drops of anti-foaming emulsion (3.5). Continue to boil vigorously until the water has been almost completely evaporated. Reduce the heat and carefully eliminate the last traces of water. When the solution becomes clear and colourless (or light green if a copper-based catalyst is used), continue to boil for another hour. Leave to cool.

5.3 Distillation and titration
Proceed as indicated in 5.2 and 5.3 of the method for the determination of crude protein (Method 5.1).
5.4 Blank test
Carry out a blank test applying the same procedure but omitting the sample to be analysed.
6. Calculation of results
Subtract the volume of sulphuric acid neutralised in the blank test from that neutralised by the test sample. 1 ml of sulphuric acid 0.1 N corresponds to 1.4 mg of nitrogen.
Multiply the quantity of nitrogen by the factor 6.25. Express the result as a percentage of the sample.
7. Repeatability
The difference between the results of two parallel determinations carried out on the same sample must not exceed:
— 0.4%, in absolute value, for contents of less than 20%;
— 2.0%, relative to the higher result, for contents of not less than 20% and not more than 40%.
—0.8%, in absolute value, for contents of more than 40%.
8. Observations
8.1 The values obtained by this method have no direct connection with digestibility in vivo.
8.2 Products with an oil or fat content exceeding 10% must first be defatted by extraction with petroleum ether (boiling point 40-60°C).
5.3 ESTIMATION OF PEPSIN ACTIVITY.
1. Purpose and scope
To establish the activity of the pepsin used in the determination of crude protein dissolved by pepsin and hydrochloric acid.
2. Principle
Haemoglobin is treated with pepsin in a hydrochloric acid medium under defined conditions. The non-hydrolysed fraction of the protein is precipitated in trichloroacetic acid. Sodium hydroxide and Folin-Ciocalteu reagent are added to the filtrate. The optical density of this solution is measured at 750 nm and the corresponding quantity of tyrosine is read from a calibration curve.
Definition: The unit of pepsin is defined as being the quantity of that enzyme which, under the conditions of the method, liberates per minute, a quantity of hydroxyaryl groups which, when stained with the Folin-Ciocalteu reagent, has an optical density corresponding to that of one μmole of tyrosine stained in the same manner.
3. Reagents
3.1 Hydrochloric acid, 0.2 N.
3.2 Hydrochloric acid, 0.06 N.
3.3 Hydrochloric acid, 0,025 N.
3.4 Trichloroacetic acid, solution, 5% (w/v).
3.5 Sodium hydroxide solution 0.5 N.
3.6 Folin-Ciocalteu reagent: place 100 g of sodium tungstate ($Na_2WO_4.2H_2O$), 25 g of sodium molybate ($Na_2Mo_04.2H_2O$) and 700 ml of water in a 2 litre round-bottomed flask fitted with a standard ground-glass joint. Add 50 ml of phosphoric acid (d : 1.71) and 100 ml of concentrated hydrochloric acid (d : 1.19), connect a reflux condenser to the flask, bring to the boil and keep the solution gently boiling for 10 hours. Leave to cool, detach the reflux condenser, add 175 g of lithium sulphate ($Li_2SO_4.2H_2O$), 50 ml

of water and 1 ml of bromine. Boil for 15 minutes to eliminate excess bromine. Leave to cool, transfer the solution to a 1 litre graduated flask, make up to volume with water, homogenize and filter. No greenish colouration must remain. Before use, dilute 1 volume of the reagent with 2 volumes of water.

3.7 Haemoglobin solution: weigh a quantity of haemoglobin (approx. 2 g of protein substratum determined according to Anson) corresponding to 354 mg of nitrogen1 and place in a 200 ml flask fitted with a standard ground-glass joint. Add a few ml of hydrochloric acid (3.2), connect the flask to the vacuum pump and shake until the haemoglobin has completely dissolved. Release the vacuum and, while shaking, add hydrochloric acid (3.2) to make up to 100 ml. Prepare immediately before use.

3.8 Standard tyrosine solution: dissolve 181.2 mg of tyrosine in the hydrochloric acid (3.1) and make up to 1 litre with the same acid (stock solution). Take 20.0 ml and dilute to 100 ml with hydrochloric acid (3.1). 1 ml of this solution contains 0.2 µmole of tyrosine.

4. Apparatus

4.1 Water bath set at 25°C ± 0.1°C.

4.2 Spectrophotometer.

4.3 Chronometer, accuracy: 1 second.

4.4 pH-meter.

5. Procedure

5.1 Preparation of the solution (see item 7.1).

Dissolve 150 mg of pepsin in 100 ml of hydrochloric acid (3.2). Pipette 2 ml of the solution into a 50 ml graduated flask and make up to volume with hydrochloric acid (3.3). The pH, checked with the pH-meter, must be 1.6±0.1. Immerse the flask in the water bath (4.1).

5.2 Hydrolysis.

Pipette 5.0 ml of haemoglobin solution (3.7) into a test tube, heat to 25°C in the water bath (4.1), add 1.0 ml of the pepsin solution obtained in 5.1 and mix with a glass rod thickened at one end, with about 10 back-and-forth movements. Leave the test tube in the water bath at 25°C for exactly 10 minutes, timed from the addition of the pepsin solution (duration and temperature must be strictly observed). Then add 10.0 ml of trichloroacetic acid solution (3.4) previously heated to 25°C, mix and filter through a dry filter.

1Determine the nitrogen content by the semi-micro Kjeldahl method (theoretical content: 17.7% of nitrogen).

5.3 Development of colouration and measurement of optical density.

Pipette 5.0 ml of the filtrate into a 50 ml Erlenmeyer flask. Add 10.0 ml of sodium hydroxide solution (3.5) and, shaking constantly, 3.0 ml of dilute Folin-Ciocalteu reagent (3.6). After 5-10 minutes, determine the optical density of the solution with the spectrophotometer at 750 nm in cells 1 cm thick against water.

5.4 Blank test

For each determination, carry out a blank test as follows:

Pipette 5.0 ml of haemoglobin solution (3.7) into a test tube, heat to 25°C in the water bath (4.1), add 10.0 ml of trichloroacetic acid solution (3.4) previously heated to 25°C, mix, then add 1.0 ml of the pepsin solution obtained in 5.1. Mix with a glass rod and leave the test tube in the water bath (4.1) at 25°C for exactly

10 minutes. Mix and filter through a dry filter. Follow the procedure indicated in 5.3.

5.5 Calibration curve.

Place 1.0, 2.0, 3.0, 4.0 and 5.0 ml aliquots of standard tyrosine solution (3.8), corresponding to 0.2, 0.4, 0.6, 0.8 and 1.0 vmoles of tyrosine respectively in 50 ml Erlenmeyer flasks. Complete the series with a reference solution free from tyrosine. Make up the volumes to 5.0 ml with hydrochloric acid (3.1). Add 10.0 ml of sodium hydroxide solution (3.5) and, shaking constantly, 3.0 ml of dilute Folin-Ciocalteu reagent (3.6). Measure the optical density as indicated in the last sentence of 5.3. Trace the calibration curve by plotting the optical densities against the quantities of tyrosine.

6. Calculation of results

From the calibration curve read the quantity of tyrosine, in vmoles, corresponding to the optical density of the coloured solution, corrected on the basis of the blank value.

The pepsin activity, in vmoles, of tyrosine at 25°C, per mg and per minute, is calculated by using the formula:

Units per mg(U/mg)=

where:

a = quantity of tyrosine, in vmoles, read from the calibration curve;

p = weight in mg of the quantity of pepsin added in 5.2.

7. Observations

7.1 The quantity of pepsin to be dissolved must be such that, on final photometric measurement, an optical density of 0.35±0.035 is obtained.

7.2 Two units per mg obtained by this method correspond to: 3.64 Anson milliunits/mg (vmoles of tyrosine/mg/min at 35.5°C) or 36,400 commercial units/g (vmoles of tyrosine/g in 10 min at 35.5°C).

5.4 DETERMINATION OF MILK POWDER.

1. Purpose and scope

To determine the skim milk powder content of milk replacers. Large quantities of buttermilk and/or of certain non-milk proteins may lead to interferences.

2. Principle

The casein is extracted with sodium citrate solution and precipitated as para-casein by rennet, following adjustment of the calcium ion concentration. The nitrogen content of the para-casein is determined according to the method for the determination of crude protein (see method 5.1). The skim milk powder content is calculated on the basis of a minimum casein content of 27.5%.

3. Reagents

3.1 Trisodium citrate, dihydrate (1% w/v solution).

3.2 Calcium chloride (saturated aqueous solution at 20°C). Dissolve about 90 g of anhydrous calcium chloride in 100 ml of distilled water by warming and slightly stirring. Leave overnight at 20°C (if a deposit of crystals is not formed, add more calcium chloride and repeat the procedure), filter the liquid next day and store the filtered solution in a well closed bottle at 20°C.

3.3 0.1 N sodium hydroxide.

3.4 0.1 N hydrochloric acid.

3.5 Liquid calf rennet (Standard strength of 1 : 10,000). Store in a refrigerator at 4 to 6°C.

3.6 All reagents listed under 3 in method 5.1 determination of crude protein.

4. Apparatus

4.1 Centrifuge (2,000 to 3,000 rpm).

4.2 Magnetic stirrer.

4.3 Thermostatically-controlled water bath at 37°C.

4.4 pH meter.

4.5 Kjeldahl digestion and distillation apparatus.

5. Procedure

5.1 Dissolution of the casein

Weigh, to the nearest 2 mg, 1 g of sample directly into a 50 ml centrifuge tube, add 30 ml of anaqueous solution of trisodium citrate (3.1) previously heated to 45°C. Mix with the aid of the magnetic stirrer (4.2) for at least five minutes. Centrifuge at 500 g (2,000 to 3,000 rpm) for 10 minutes and decant the clear aqueous supernatant into a 150 to 200 ml beaker. Ensure that the sediment remains in the centifuge tube. Carry out two further extractions on the residue, according to the same procedure, and combine the extracts. If a layer of oil forms at the surface, cool in a refrigerator until the fat solidifies and remove the solid layer with a spatula.

5.2 Coagulation of casein with the enzymes of rennet.

While stirring continuously, add dropwise 1 ml of a saturated solution of calcium chloride (3.2) to the total aqueous extract (about 100 ml). Adjust the pH to 6.4-6.5 with solutions of NaOH (3.3) or HCl (3.4) Place in the thermostatically controlled water bath at 37°C for 15 to 20 minutes to obtain saline balance. This becomes more evident by the formation of a light turbidity. (If a precipitate forms it must be removed by further centrifugation at 1,000 rpm for five minutes and the supernatant is decanted without washing the sediment). Remove the extract from the water bath and immediately add 0.5 ml of liquid rennet (3.5) dropwise, with constant stirring. Coagulation appears in one to two minutes. Leave at a temperature of 20 to 37°C for 10 to 15 minutes and break the coagulum by stirring. Transfer the casein quantitatively on to a fast ashless filter while washing three times with 15 ml of distilled water (3 x 15 ml). Filtration must be completed within two hours.

5.3 Determination of casein nitrogen

The filter paper and precipitate is placed in a Kjeldahl flask and nitrogen is determined by the Kjeldahl method as described in method 5.1 determination of crude protein.

5.4 Blank test

Carry out a blank test applying the same procedure but omitting the sample to be analysed.

6. Calculation of results

Subtract the volume of sulphuric acid neutralised in the blank test from that neutralised by the test sample. 1 ml of sulphuric acid (0. 1 N) corresponds to 1.4 mg of nitrogen. The percentage of skimmed-milk powder in the compound feedingstuff is calculated by the following formula:

where N is the percentage of para-casein nitrogen; 27.5 is the factor for converting determined casein into a percentage of skimmed-milk powder.

7. Repeatability
1.0% relative to the higher result.
5.5 DETERMINATION OF VOLATILE NITROGENOUS BASES
5.5.1 Microdiffusion Method
1. Purpose and scope
To determine the content of volatile nitrogeneous 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.
3. Reagents
3.1 Trichloroacetic acid solution 20% (w/v).
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 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 vg of ammonia.
3.4 Potassium carbonate saturated solution: dissolve 100 g of potassium carbonate in 100 ml of boiling water. Cool and filter.
3.5 Sulphuric acid 0.02 N.
4. Apparatus
4.1 Mixer (tumbler): approximately 35 to 40 rpm.
4.2 Glass or plastic Conway cells (see diagram).
4.3 Microburettes graduated in 1/100 ml.
5. Procedure
Weigh to the nearest mg, approximately 10 g of sample and place in a 200 ml graduated flask. Add 100 ml water and mix in the tumbler (4.1) for 30 mins. Add 50 ml of trichloroacetic acid solution (3.1), make up to volume with water, shake vigorously and filter. If the ammonia content of the sample exceeds 0.6% dilute an aliquot before proceeding to the next stage.
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°C.
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 sulphuric acid 0.02 N corresponds to 0.34 mg of ammonia.
Express the result as a percentage of the sample.
7. Repeatability
The difference between the results of two parallel determinations carried out on the same sample should not exceed:

10% relative to the higher value, for ammonia contents less than 1.0%.
0.1% in absolute value for ammonia contents equal to or greater than 1.0%.

5.5 DETERMINATION OF VOLATILE NITROGENOUS BASES
5.5.2 Distillation method
1. Purpose and scope
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 Trichloroacetic acid solution 20% (w/v).
3.2 Magnesium oxide.
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 Methyl red indicator; dissolve 300 mg of methyl red in 100 ml of 95-96% (v/v) ethanol.
4. Apparatus
4.1 Mixer (tumbler): approximately 35 to 40 rpm.
4.2 Distilling apparatus of the Kjeldahl type.
5. Procedure
Weigh to the nearest mg, approximately 10 g of sample and place it in a 200 ml graduated flask. Add 100 ml of water and mix in the tumbler (4.1) for 30 mins. 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 sulphuric acid 0.1 N corresponds to 1.7 mg of ammonia.
7. Repeatability
The difference between the results of two parallel determinations carried out on the same sample should not exceed, in relative value, 10% of ammonia.
6. MINERAL SUBSTANCES.

6.1 DETERMINATION OF CRUDE ASH.

1. Purpose and scope

To determine the crude ash content of feeding stuffs.

2. Principle

The sample is ashed at 550°C; the residue is weighed.

3. Reagent

Ammonium nitrate solution, 20% (w/v).

4. Apparatus

4.1 Hot plate.

4.2 Electric muffle-furnace with thermostat.

4.3 Crucibles for ashing made of platinum or an alloy of platinum and gold, or silica or porcelain.

5. Procedure

Weigh, to the nearest mg, approximately 5 g of the sample (2.5 g in the case of products which have a tendency to swell) and place in a crucible for ashing (4.3) which has first been heated to 550°C ± 5°C, cooled in a "desiccator" and weighed. Place the crucible on the hot plate (4.1) and heat gradually until the substance carbonises. Put the crucible into the muffle-furnace (4.2) set at 550°C ± 5°C. Keep at this temperature until white, light grey or reddish ash is obtained which appears to be free from carbonaceous particles. Place the crucible in a desiccator, leave to cool and weigh immediately.

6. Calculation of results

Calculate the weight of the residue and express the result as a percentage of the sample.

7. Observations

7.1 Substances which are difficult to ash must be subjected to an initial ashing of at least 3 hours, cooled and then a few drops of 20% solution of ammonium nitrate (3) added to it (carefully, to avoid dispersal of the ash or the formation of lumps). Continue ashing after drying in the oven. Repeat the operation as necessary until ashing is complete.

7.2 In the case of substances resistant to the treatment described under 7.1, proceed as follows: after ashing for 3 hours, place the ash in warm water and filter through a small, ash-free filter. Ash the filter and its contents in the original crucible. Place the filtrate in the cooled crucible, evaporate until dry, ash and weigh.

7.3 In the case of oils and fats, weigh accurately a sample of approximately 25 g in a suitably sized crucible. Carbonise by setting light to the substance with a strip of ash-free filter paper. After combustion, moisten with as little water as possible. Dry and ash as described under 5.

6.2 DETERMINATION OF ASH WHICH IS INSOLUBLE IN HYDROCHLORIC ACID.

1. Purpose and scope

To determine the content, in feeding stuffs, of mineral substances which are insoluble in hydrochloric acid. Ether of two methods can be used, depending on the nature of the sample.

1.1 Method A: applicable to straight organic feeding stuffs and to compound feedingstuffs other than those specified under Method B.

1.2 Method B: applicable to mineral compounds and mixtures and to compounds feeding stuffs whose content of substances insoluble in hydrochloric acid, as determined by Method A, is greater than 1%.

2. Principle

2.1 Method A: the sample is ashed, the ash boiled in hydrochloric

acid and the insoluble residue filtered and weighed.
2.2 Method B: the sample is treated with hydrochloric acid. The solution is filtered, the residue ashed and the ash thus obtained treated in accordance with Method A.
3. Reagents
3.1 Hydrochloric acid solution, 3 N.
3.2 Trichloroacetic acid solution, 20% (w/v).
3.3 Trichloroacetic acid solution, 1% (w/v).
4. Apparatus
4.1 Hot plate.
4.2 Electric muffle-furnace with thermostat.
4.3 Crucibles for ashing made of platinum or an alloy of platinum and gold, or of silica or porcelain.
5. Procedure
5.1 Method A:
Ash the sample using the method prescribed in this schedule for the determination of crude ash. Ash obtained from that analysis may also be used.
Place the ash in a 250-400 ml beaker using 75 ml of hydrochloric acid 3 N (3.1). Bring slowly to the boil and boil gently for 15 minutes. Filter the warm solution through an ash-free filter paper and wash the residue with warm water until the acid reaction is no longer visible. Dry the filter containing the residue and ash in a tared crucible at a temperature of not less than 550°C and not more than 700°C. Cool in a desiccator and weigh.
5.2 Method B:
Weigh, to the nearest mg, approximately 5 g of the sample, and place in a 250-400 ml beaker. Add 25 ml of water and 25 ml of hydrochloric acid 3 N (3.1) successively, mix and wait for effervescence to cease. Add a further 50 ml of hydrochloric acid 3 N (3.1). Wait for any release of gas to cease then place the beaker in a boiling water bath and keep it there for 30 minutes (or longer if necessary) in order to hydrolyse thoroughly any starch which may be present.
Filter while warm through an ash-free filter and wash the filter in 50 ml of warm water (see observation, 7). Place the filter containing the residue in a crucible for ashing (4.3), dry and ash at a temperature of not less than 550°C and not more than 700°C. Place the ash in a 250-400 ml beaker using 75 ml of hydrochloric acid 3 N (3.1); continue as described in the second sub-paragraph of 5.1.
6. Calculation of results
Calculate the weight of the residue and express the result as a percentage of the sample.
7. Observation
If filtration proves difficult recommence the analysis, replace the 50 ml of hydrochloric acid 3 N (3.1) by 50 ml of 20% trichloroacetic acid (3.2) and washing the filter in a warm solution of 1% trichloroacetic acid (3.3).
6.3 DETERMINATION OF WATER-SOLUBLE CHLORIDES.
1. Purpose and scope
To determine the content of water-soluble chlorides, expressed as sodium chlorides, in all feeding stuffs and mineral mixtures.
2. Principle
The chlorides are dissolved in water. If the product contains

organic matter it is clarified. The solution is slightly acidified with nitric acid and the chlorides precipitated in the form of silver chloride by means of a solution of silver nitrate. The excess silver nitrate is titrated with a solution of ammonium thiocyanate, by Volhard's method.

3. Reagents

3.1 Ammonium thiocyanate solution, 0.1 N.

3.2 Silver nitrate solution, 0.1 N.

3.3 Ammonium ferric sulphate, saturated solution.

3.4 Nitric acid, d : 1.38.

3.5 Diethyl ether or light petroleum, boiling range 40-60°C.

3.6 Acetone.

3.7 Carrez I solution: dissolve in water 21.9 g of zinc acetate (Zn $CH_3COO)_2.2H_2O$) and 3 g of glacial acetic acid. Make up to 100 ml with water.

3.8 Carrez II solution: dissolve in water 10.6 g of potassium ferrocyanide, $K_4[Fe(CN)_6].3H_2O$. Make up to 100 ml with water.

3.9 Active carbon, free from chlorides and not absorbing them.

4. Apparatus

Mixer (tumbler): approximately 35-40 rpm.

5. Procedure

5.1 Preparation of the solution.

According to the nature of the sample, prepare a solution as shown under 5.1.1, 5.1.2 or 5.1.3.

At the same time carry out a blank test omitting the sample to be analysed.

5.1.1 Samples free from organic matter.

Weigh, to the nearest mg, a sample of not more than 10 g and containing not more than 3 g of chlorine in the form of chlorides. Place with 400 ml of water in a 500 ml volumetric flask at approximately 20°C. Mix for 30 minutes in the tumbler, bring up to volume, mix and filter.

5.1.2 Samples containing organic matter, excluding the products listed under 5.1.3.

Weigh, to the nearest mg, approximately 5 g of the sample and place with 1 g of active carbon (3.9) in a 500 ml volumetric flask. Add 400 ml of water at approximately 20°C and 5 ml of Carrez solution I (3.7), stir, then add 5 ml of Carrez solution II (3.8). Mix for 30 minutes in the tumbler, bring up to volume, mix and filter.

5.1.3 Cooked feeding stuffs, linseed cakes and meal, products rich in linseed meal and other products rich in mucilage or in colloidal substances (for example, dextrinated starch).

Prepare the solution as described under 5.1.2 but do not filter. Decant (if necessary centrifuge), remove 100 ml of the supernatant liquid and transfer to a 200 ml measuring flask. Mix with acetone (3.6) and bring up to volume with this solvent, mix and filter.

5.2 Titration

Using a pipette, transfer to an Erlenmeyer flask from 25-100 ml of the filtrate (according to the assumed chlorine content) obtained as described under 5.1.1, 5.1.2 or 5.1.3. The aliquot portion must not contain more than 150 mg of chlorine (Cl). Dilute if necessary to not less than 50 ml with water, add 5 ml of nitric acid (3.4), 20 ml of saturated solution of ammonium ferric sulphate (3.3) and two drops of ammonium thiocyanate solution (3.1) transferred by means

of a burette filled up to the zero mark. Using a burette, transfer the silver nitrate solution (3.2) in such a way that an excess of 5 ml is obtained. Add 5 ml of diethyl ether or light petroleum (3.5) and shake hard to coagulate the precipitate.
Titrate the excess silver nitrate with the ammonium thiocyanate solution (3.1) until the reddish-brown tint has lasted for 1 minute.
6. Calculation of results.
The amount of chlorine (W), expressed as sodium chloride, present in the volume of filtrate taken for titration is calculated by using the following formula:
$W=5.845(V1-V2)$ mg
where:
V1=ml of silver nitrate solution 0.1 N added;
V2=ml of ammonium thiocyanate solution 0.1 N used for titration.
If the blank test indicates that silver nitrate solution 0.1 N has been consumed deduct this value from the volume (V1-V2).
7. Observations
7.1 Titration may also be carried out by potentiometry.
7.2 In the case of products which are very rich in oils, first defat with light petroleum.
7.3 In the case of fish meal, titration may be carried out by Mohr's method.
6.4 DETERMINATION OF CARBONATES.
1. Purpose and scope
To determine the content of carbonates, conventionally expressed as calcium carbonate, in most feedingstuffs.
However in certain cases (for example, with iron carbonate) a special method must be used.
2. Principle
The carbonates are decomposed in hydrochloric acid; the carbon dioxide released is collected in a graduated tube and its volume compared with that released under the same conditions by a known quantity of calcium carbonate.
3. Reagents
3.1 Hydrochloric acid, d : 1.10.
3.2 Calcium carbonate.
3.3 Sulphuric acid, approximately 0.1 N, coloured with methyl red.
4. Apparatus
Scheibler-Deitrich apparatus (see diagram fig. 1) or equivalent apparatus.
5. Procedure
According to the sample's carbonate content, weigh a portion of the sample as shown below:
0.5 g for products containing from 50-100 per cent of carbonates, expressed as calcium carbonate;
1 g for products containing from 40-50 per cent of carbonates, expressed as calcium carbonate;
2-3 g for other products.
Place the portion of the sample in the special flask (4) of the apparatus, fitted with a small tube of unbreakable material containing 10 ml of hydrochloric acid (3.1), and connect the flask to the apparatus. Turn the three-way cock (5) so that the tube (1) connects with the outside. Using the mobile tube (2) which is filled with coloured sulphuric acid (3.3) and connected to the graduated tube (1), bring the level of the liquid up to the zero

mark. Turn the cock (5) in order to connect up tubes (1) and (3) and check that the level is at zero.
Run the hydrochloric acid (3.1) slowly over the portion of the sample, tilting the flask (4). Make the pressure equal by lowering the tube (2). Shake the flask (4) until the release of carbon dioxide has stopped completely.
Restore pressure by bringing the liquid back to the same level in tubes (1) and (2). After a few minutes, when the volume of gas has become constant, take the reading.
Carry out a control test in the same conditions on 0.5 g of calcium carbonate (3.2).
6. Calculation of results
The content in grams of carbonates, expressed as calcium carbonate, as a percentage of the sample, is calculated by using the formula:

where:
V=ml of CO_2 released by the portion of the sample;
T=ml of CO_2 released by 0.5 g of $CaCO_3$;
W=weight, in grams, of the portion of the sample.
7. Observations
7.1 When the portion of the sample weighs more than 2 g first place 15 ml of water in the flask (4) and mix before beginning the test. Use the same volume of water for the control test.
7.2 If the apparatus used has a different volume from that of the Schiebler-Dietrich apparatus, the portions taken from the sample and from the control substance and the calculation of the results must be adapted accordingly.
6.5 DETERMINATION OF CALCIUM.
1. Purpose and scope
To determine the total calcium content of feeding stuffs.
2. Principle
The sample is ashed, the ash treated with hydrochloric acid and the calcium precipitated as calcium oxalate. The precipitate is dissolved in sulphuric acid and the oxalic acid formed is titrated with a solution of potassium permanganate.
3. Reagents
3.1 Hydrochloric acid, d : 1.14.
3.2 Nitric acid, d : 1.40.
3.3 Sulphuric acid, d : 1.13.
3.4 Ammonia, d : 0.98.
3.5 Cold saturated solution of ammonium oxalate.
3.6 Citric acid solution. 30% (w/v).
3.7 Ammonium chloride solution, 5% (w/v).
SCHEIBLER—DIETRICH APPARATUS FOR DETERMINATION OF CO_2

Scale 1/8
(measured in mm)
Fig. 1.
3.8 Bromocresol green solution, 0.04% (w/v).
3.9 Potassium permanganate solution, 0.1 N.
4. Apparatus
4.1 Electric muffle-furnace with air circulation and thermostat.
4.2 Platinum, silica or porcelain crucibles for ashing.
4.3 Glass filter crucibles of G4 porosity.
5. Procedure

Weigh, to the nearest mg, approximately 5 g of the sample (or more if necessary), incinerate at 550C and transfer the ash to a 250 ml beaker.
Add 40 ml of hydrochloric acid (3.1), 60 ml of water and a few drops of nitric acid (3.2). Bring to the boil and keep at boiling point for 30 minutes. Cool and transfer the solution to a 250 ml volumetric flask. Rinse, bring the volume up to the mark with water, mix and filter.
Using a pipette, transfer to a 250 ml beaker an aliquot containing 10-40 mg of calcium according to the assumed calcium content. Add 1 ml of citric acid solution (3.6) and 5 ml of ammonium chloride solution (3.7).
Make the volume up to approximately 100 ml with water. Bring to the boil, add eight to ten drops of bromocresol green solution (3.8) and 30 ml of a warm solution of ammonium oxalate (3.5). If a precipitate forms, dissolve it by adding a few drops of hydrochloric acid (3.1).
Neutralise very slowly with ammonia (3.4), stirring continuously until a pH reading of 4.44.6 is obtained (i.e. when the indicator changes colour). Place the beaker in a boiling water bath and keep there for 30 minutes to allow the precipitate which has formed to settle. Remove the beaker from the water bath. Leave it to stand for an hour and filter through a G4 filter crucible (4.3).
Wash the beaker and the crucible with water until the excess ammonium oxalate is completely removed (the absence of chloride in the washing water indicates that they have been sufficiently washed). Dissolve the precipitate on the filter in 50 ml of warm sulphuric acid (3.3). Rinse the crucible with warm water and make the filtrate up to approximately 100 ml. Bring the temperature up to 70-80C and titrate drop by drop with a solution of potassium permanganate (3.9) until a pink colour is obtained which lasts for 1 minute.
6. Calculation of results
1 ml of potassium permanganate 0.1 N corresponds to 2.004 mg of calcium. Express the result obtained as a percentage of the sample.
7. Observations
7.1 For very low contents of calcium proceed as follows: filter the calcium oxalate precipitate through an ash-free filter paper. After washing, dry the filter and ash at 550C in a platinum crucible. Redissolve the residue in a few drops of sulphuric acid (3.3), evaporate until dry, incinerate again at 550C and weigh. If W is the weight of the calcium sulphate obtained, the calcium content of the aliquot amount taken as a sample=W.0.2944.
7.2 If the sample consists solely of mineral substances, dissolve in hydrochloric acid (3.1) without ashing it first. In the case of products such as calcium aluminium phosphate which are difficult to dissolve in acid, melt as follows by an alkaline process before dissolving: mix the sample to be analysed thoroughly in a platinum crucible with a mixture five times its weight, consisting of equal amounts of potassium carbonate and sodium carbonate. Heat carefully until the mixture is completely melted. Cool and dissolve in hydrochloric acid.
7.3 If the magnesium content of the sample is high, precipitate the calcium oxalate a second time.
6.6 DETERMINATION OF MAGNESIUM.

6.6.1 Atomic absorption method.
1. Purpose and scope
To determine the magnesium content of feeding stuffs. It is particularly appropriate for determining magnesium contents lower than 5%.
2. Principle
The sample is ashed and dissolved in dilute hydrochloric acid. If it contains no organic substances, it is dissolved directly in dilute hydrochloric acid. The solution is diluted and the magnesium content determined by atomic absorption spectrophotometry at 285.2 nm, by comparison with standard solutions.
3. Reagents
3.1 Hydrochloric acid, d : 1.16.
3.2 Hydrochloric acid, d : 1.19.
3.3 Magnesium ribbon or wire, or magnesium sulphate heptahydrate, dried at room temperature.
3.4 Strontium salt solution (chloride or nitrate) at 2.5% (w/v) strontium (=76.08 g $SrCl_2.6H_2O$ or 60.38 g $Sr(NO_3)_2$ per 1000 ml).
3.5 Standard magnesium solution: weigh, to the nearest mg, 1 g magnesium (3.3) which has previously had its oxide coating carefully removed, or the corresponding quantity (10.143 g) of magnesium sulphate heptahydrate (3.3). Place in a 1000 ml graduated flask, add 80 ml hydrochloric acid (3.1), leave to dissolve and make up to 1000 ml with water. 1 ml of this solution contains 1.000 mg magnesium.
4. Apparatus
4.1 Platinum, silica or porcelain ashing crucibles.
4.2 Thermostatically controlled electric muffle-furnace.
4.3 Atomic absorption spectrophotometer.
5. Procedure
5.1 Preparation of the sample solution.
5.1.1 Feeding stuffs composed exclusively of mineral substances.
Weigh, to the nearest mg, approximately 5 g of the sample into a 500 ml graduated flask with 250-300 ml water. Add 40 ml hydrochloric acid (3.1), bring to the boil and keep the liquid gently boiling for 30 minutes. Leave to cool, make up to volume with water, mix and filter into a dry beaker through a dry pleated filter. Discard the first 30 ml of the filtrate. In the presence of silica, treat 5 g of sample with a sufficient quantity (15-30 ml) of hydrochloric acid (3.2), evaporate to dryness on a water bath and transfer to an oven at 105C for 1 hour. Proceed as from the third sentence of 5.1.2.
5.1.2 Feeding stuffs composed predominantly of mineral substances.
Weigh, to the nearest mg, approximately 5 g of the sample into a crucible (4.1) and ash at 550C in the muffle-furnace (4.2) until an ash which is free from carbonaceous particles is obtained, and leave to cool. In order to eliminate silica, add to the ash a sufficient quantity (15-30 ml) of hydrochloric acid (3.2), evaporate to dryness on a water bath and transfer to an oven at 105ºC for 1 hour. Treat the residue with 10 ml hydrochloric acid (3.1) and transfer to a 500 ml graduated flask using warm water. Leave to cool and make up to volume with water. Mix and filter into a dry beaker through a dry pleated filter. Discard the first 30 ml of the filtrate.
5.1.3 Feeding stuffs composed predominantly of organic substances.

Weigh, to the nearest mg, approximately 5 g of the sample into a crucible (4.1) and ash at 550C in the muffle-furnace (4.2) until an ash which is free from carbonaceous particles is obtained. Treat the ash with 5 ml hydrochloric acid (3.2), evaporate to dryness on a water bath and then dry for 1 hour in the oven at 105C in order to render the silica insoluble. Treat the ash with 5 ml hydrochloric acid (3.1), transfer to a 250 ml graduated flask using warm water, bring to the boil, leave to cool and make up to volume with water. Mix and filter into a dry beaker through a dry pleated filter. Discard the first 30 ml of the filtrate.

5.2. Measurement by atomic absorption

By diluting the standard solution (3.5) with water, prepare at least 5 reference solutions of increasing concentration, corresponding to the optimal measuring range of the spectrophotometer (4.3). Add to each solution 10 ml strontium salt solution (3.4) and then make up the volume to 100 ml with water. Dilute with water one aliquot of the filtrate obtained from 5.1.1, 5.1.2 or 5.1.3, so as to obtain a magnesium concentration which is within the limits of concentration of the reference solutions. The hydrochloric acid concentration of this solution must not exceed 0.4 N. Add 10 ml strontium salt solution (3.4) and then make up the volume to 100 ml with water. Measure the absorption of the solution to be determined and of the reference solutions at 285.2 nm.

6. Calculation of results

Calculate the quantity of magnesium in the sample by relation to the reference solutions. Express the result as a percentage of the sample.

7. Repeatability

The difference between the results of two parallel determinations carried out on the same sample must not exceed 5%, relative to the higher result.

6.7 DETERMINATION OF TOTAL PHOSPHORUS.

6.7.1 Spectrophotometric method.

1. Purpose and scope

To determine the total phosphorus content of feeding stuffs and mineral mixtures low in phosphorus.

2. Principle

The sample is mineralised, either by dry combustion (in the case of organic feeding stuffs) or by acid digestion (in the case of mineral compounds and liquid feeding stuffs), 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.

3.2 Hydrochloric acid d : 1.1 (approximately 6 N).

3.3 Nitric acid, d : 1.045.

3.4 Nitric acid, d : 1.38-1.42.

3.5 Sulphuric acid, 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 (NH4)6Mo7O244H2O. Add 10 ml of ammonia (d

: 0.91) and make up to 1 litre with water.
3.6.2 Ammonium monovanadate solution: dissolve 2.35 g of ammonium monovanadate (NH_4VO_3) 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 (KH_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 550C.
4.3 Kjeldahl flask.
4.4 Graduated flasks and precision pipettes.
4.5 Spectrophotometer.
4.6 Test tubes with stoppers.
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, to the nearest mg, 1 g or more of the sample. Place the test sample in a Kjeldahl flask, add 20 ml of sulphuric acid (3.5), shake to impregnate the substance completely 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, mix and filter.
5.1.2 Samples containing organic substances and free from calcium and magnesium dihydrogen phosphates.
Weigh, to the nearest mg, about 2.5 g of the sample in an ashing crucible (4.1). Mix the test sample until completely merged with 1 g of calcium carbonate (3.1). Ash in the oven at 550C±5C until white or grey ash is obtained (a little charcoal does not matter). Transfer the ash into a 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 to dryness 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. 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, mix and filter.
5.2 Development of colouration 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 vg/ml. Place 10 ml of this solution in a test tube (4.6) and add 10 ml of molybdovanadate reagent (3.6). Mix and leave to stand for at least 10 minutes at 20C. Measure the optical density in a spectrophotometer (4.5), 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 vg of phosphorus per ml. Take 10 ml of each of these solutions and add 10 ml of molybdovanadate reagent (3.6). Mix and leave to stand for at least 10 minutes at 20C. 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 0-40 vg/ml, the curve will be linear.
6. Calculation of results
Determine the amount of phosphorous in the test sample by using the calibration curve. Express the result as a percentage of the sample.
7. Repeatability
The difference between results of two parallel determinations carried out on the same sample should not exceed:
3% relative to the higher result, for phosphorus contents of less than 5%; 0.15% in absolute value, for phosphorus contents equal to or greater than 5%.
6.8 DETERMINATION OF POTASSIUM.
1. Purpose and scope
To determine the content of potassium in feeding stuffs.
2. Principle
The sample is ashed and the ash dissolved in hydrochloric acid. The potassium content of the solution is determined by flame photometry in the presence of caesium chloride and aluminium nitrate. The addition of these substances largely eliminates interference from disturbing elements.
3. Reagents
3.1 Hydrochloric acid d : 1.12.
3.2 Caesium chloride.
3.3 Aluminium nitrate, $Al(NO3)3.9H2O$.
3.4 Potassium chloride, anhydrous.
3.5 Loading agent: dissolve in water 50 g of caesium chloride (3.2) and 250 g of aluminium nitrate (3.3), make up to 1 litre with water and mix. Store in plastic bottles.
3.6 Standard solution of potassium: dissolve in water 1.907 g of potassium chloride (3.4), add 5 ml of hydrochloric acid (3.1), make up to 1 litre with water and mix. Store in plastic bottles. 1 ml of this solution contains 1.00 mg of potassium.
4. Apparatus
4.1 Platinum, silica or porcelain crucibles for ashing, provided if necessary with lids.
4.2 Electric muffle-furnace with thermostat.
4.3 Flame photometer.
5. Procedure
5.1 Analysis of sample.
As a general rule, weigh, to the nearest 10 mg approximately 10 g of the sample, place in a crucible (4.1) and ash at 450C for 3 hours. After cooling, transfer the ash quantitatively to a 500 ml graduated flask using 250-300 ml of water and then 50 ml of hydrochloric acid (3.1). When all release of carbon dioxide has ceased, heat the solution and keep at a temperature of about 90C for 2 hours, stirring occasionally. After cooling to room temperature, make up to the mark with water, shake and filter.
Transfer to a 100 ml graduated flask an aliquot part of the filtrate containing a maximum of 1.0 mg of potassium, add 10.0 ml

of loading agent (3.5), make up to the mark with water and mix. In the case of higher levels of potassium dilute the solution to be analysed in suitable proportions before adding the loading agent. The table below is given as a guide for a sample of about 10 g.

Assumed potassium content of the sample (%K)Dilution factorAliquot part in ml of the solutionUp to 0.1—500.1 to 0.5—100.5 to 1.0—51.0 to 5.01 : 10105.0 to 10.01 : 10510.0 to 20.01 : 205

Measure by flame photometry at a wavelength of 768 nm. Calculate the results by means of a calibration curve.

5.2 Calibration curve.

Place exactly 10 ml of the standard solution (3.6) in a 250 ml graduated flask, make up to the mark with water and mix. Place in 100 ml graduated flasks exactly 5, 10, 15, 20 and 25 ml of this solution, corresponding respectively to amounts of potassium of 0.2, 0.4, 0.6, 0.8 and 1.0 mg. Complete the series with a blank flask containing no standard solution. Add 10 ml of loading agent (3.5) to each flask, make up to the mark with water and mix. Carry out the measurements as indicated in 5.1. The calibration curve is generally linear up to potassium concentration of 1 mg in 100 ml of solution.

6. Calculation of results.

Express the result as a percentage of the sample.

7. Observation

It is not always necessary to add loading agent (3.5) in order to eliminate the interference of disturbing elements.

6.9 DETERMINATION OF SODIUM.

1. Purpose and scope

To determine the content of sodium in feeding stuffs.

2. Principle

The sample is ashed and the ash dissolved in hydrochloric acid. The sodium content of the solution is determined by flame photometry in the presence of caesium chloride and aluminium nitrate. The addition of these substances largely eliminates interference from disturbing elements.

3. Reagents

3.1 Hydrochloric acid, d : 1.12.

3.2 Caesium chloride.

3.3 Aluminium nitrate, $Al(NO3)3.9H2O$.

3.4 Sodium chloride, anhydrous.

3.5 Loading agent: dissolve in water 50 g of caesium chloride (3.2) and 250 g of aluminium nitrate (3.3), make up to 1 litre with water and mix. Store in plastic bottles.

3.6 Standard solution of sodium: dissolve in water 2.542 g of sodium chloride (3.4) add 5 ml of hydrochloric acid (3.1), make up to 1 litre with water and mix. Store in plastic bottles. 1 ml of this solution contains 1.00 mg of sodium.

4. Apparatus

4.1 Platinum, silica or porcelain crucibles for ashing, provided if necessary with lids.

4.2 Electric muffle-furnace with thermostat.

4.3 Flame photometer.

5. Procedure

5.1 Analysis of sample

As a general rule, weigh, to the nearest 10 mg, approximately 10 g of the sample, place in a crucible (4.1) and ash at 450°C for 3 hours. Avoid overheating (ignition). After cooling, transfer the ash quantitatively to a 500 ml graduated flask, using 250-300 ml of water and then 50 ml of hydrochloric acid (3.1). When all release of carbon dioxide has ceased, heat the solution and keep at a temperature of about 90°C for 2 hours, stirring occasionally. After cooling to room temperature, make up to the mark with water, shake and filter. Transfer to a 100 ml graduated flask an aliquot part of the filtrate containing a maximum of 1.0 mg of sodium, add 10.0 ml of loading agent (3.5), make up to the mark with water and mix. In the case of higher levels of sodium, dilute the solution to be analysed in suitable proportions before adding the loading agent. Measure by flame photometry at a wavelength of 589 nm. Calculate the result by means of a calibration curve.
The table below is given as a guide for a sample of about 10 g.

Assumed sodium content of the sample (%Na)Dilution factorAliquot part in ml of
the solutionUp to 0.1—500.1 to 0.5—100.5 to 1.0—51.0 to 5.01 : 10105.0 to 10.01 : 10510.0 to 20.01 : 205
5.2 Calibration curve.
Place exactly 10 ml of the standard solution (3.6) in a 250 ml graduated flask, make up to the mark with water and mix. Place in 100 ml graduated flasks exactly 5, 10, 15, 20 and 25 ml of this solution, corresponding respectively to amounts of sodium of 0.2, 0.4, 0.6, 0.8 and 1.0 mg. Complete the series with a blank flask containing no standard solution. Add 10 ml of loading agent (3.5) to each flask, make up to the mark with water and mix. Carry out the measurements as indicated in 5.1. The calibration curve is generally linear up to a sodium concentration of 1 mg in 100 ml of solution.
6. Calculation of results
Express the result as a percentage of the sample.
7. Observations
7.1 For products containing more than 4% of sodium, it is preferable to ash the substance for 2 hours in a crucible with a lid. After cooling, add water, bring the ash into suspension by means of a platinum wire, dry and ash again for 2 hours in the crucible with a lid.
7.2 If the sample consists solely of mineral substances, dissolve without prior ashing.
7. MISCELLANEOUS.
7.1 DETERMINATION OF HECTOLITRE WEIGHT.
1. Purpose and Scope
To determine the hectolitre weight of cereals.
2. Principle
The bulk density of cereals expressed as kilogram per hectolitre, is calculated from the weight of one bushel (measured in a specified manner) of cereal. Bushel weight is converted to hectolitre weight by multiplying by the factor 1.25.
3. Apparatus
3.1 Balance and weights conforming to the weights and measures Act 1904.
3.2 Measuring cylinder made of brass or copper with a perforated

base an observation window fitted at its lower end and a slot at the upper end for the insertion and withdrawal of the levelling plate (3.3).
3.3 Levelling plate.
3.4 Auxiliary cylinder made of brass or copper.
3.5 A filling cylinder with a double funnel arrangement at its outlet.
3.6 Cylindrical drop weight.
3.7 Disk.
4. Procedure
Check that the weights pan exactly counterbalances the measuring cylinder (3.2) and the drop weight (3.6). Place the measuring cylinder (3.2) on the disk (3.7). Insert the levelling plate (3.3) into the slot at the top of the measuring cylinder (3.2) and place the drop weight (3.6) on top of it. Place auxiliary cylinder (3.4) on top of (3.2).
Fill the filling cylinder (3.5) with cereal to the level of the outlet hole (do not tap the tube). Place on top of cylinder (3.3) allow the contents to empty completely into (3.4) and then remove cylinder (3.5). Withdraw the levelling plate (3.3) completely and reinsert after the drop weight (3.6) has fallen to the bottom of the measuring cylinder (3.2). Remove the auxiliary cylinder (3.4) and discard the surplus cereal remaining above the levelling plate (3.3). Remove the levelling plate (3.3) and weigh the measuring cylinder (3.2) together with the drop weigh (3.6) and the cereal, to obtain the bushel weight.
5. Calculation of results
To convert bushel weight to hectolitre weight, multiply the weight obtained in the preceding paragraph by 1.25.
6. Repeatability
The difference in results between two determinations carried out simultaneously or in rapid succession on the same sample, by the same analyst, under the same conditions, shall not exceed 0.1% in absolute value.
7.2 DETERMINATION OF TOTAL SOLIDS.
1. Purpose and Scope
To determine the total solids content of milk products.
2. Principle
A known quantity of sample is dried at a constant temperature and to a constant weight. The residue after drying constitutes the total solids.
3. Apparatus
3.1 Non-corrodible, flat metal dishes about 2 cm deep and approximately 6–8 cm in diameter with well fitting lids.
3.2 Air drying oven at 102°C±2°C.
3.3 Water bath.
4. Procedure
Place dish (3.1) in a drying oven (3.2) for approximately 30 minutes. Cool in a dessicator and weigh accurately. Weigh to the nearest mg approximately 3 g of sample directly into the dish and place the uncovered dish on a boiling water bath for 30 minutes. Transfer to an oven (3.2) and leave for 2 hours. Remove to a desiccator, cool and weigh. Dry in the oven (3.2) for a further hour, cool and weigh. Repeat the drying and weighing until the difference between two successive weighings is not more than 0.5 mg.

5. Calculation of results
Express the result as a percentage of the sample.
7.3 DETERMINATION OF THE UREASE ACTIVITY OF PRODUCTS DERIVED FROM SOYA BEANS.
1. Purpose and scope
To estimate the urease activity of products derived from soya beans and to show whether these products have been cooked for a sufficient length of time.
2. Principle
Urease activity is estimated by the amount of ammoniacal nitrogen liberated per 1 g of product per minute at 30°C from a solution of urea.
3. Reagents
3.1 Hydrochloric acid, 0.1 N.
3.2 Sodium hydroxide solution, 0.1 N.
3.3 Loading agent of phosphate 0.05 M, containing, per 1000 ml, 4.45 g of disodium phosphate ($Na_2HPO_4.2H_2O$) and 3.40 g of monopotassium phosphate (KH_2PO_4).
3.4 Freshly-prepared urea loading agent containing 30.0 g of urea per 1000 ml of loading agent (3.3); pH 6.9-7.0.
4. Apparatus
4.1 Potentiometric titration apparatus or high sensitivity pH-meter (0.02 pH) with magnetic stirrer.
4.2 Water bath fitted with thermostat set at 30°C exactly.
4.3 Test tubes with ground glass stoppers.
5. Procedure
Crush about 10g of the sample (e.g. in a coffee mill) so that it passes through a sieve with a mesh of 0.2 mm. Weigh 0.2 g of the crushed sample to the nearest mg, place in a test tube with a ground glass stopper and add 10 ml of urea loading agent (3.4). Stopper immediately and shake vigorously. Place the tube in a water bath (4.2), set at 30°C exactly and keep there for exactly 30 minutes. Immediately add 10 ml of 0.1 N hydrochloric acid (3.1) cool rapidly to 20°C and transfer the contents of the tube quantitatively to a titration vessel, rinsing twice with 5 ml of water. Using a glass electrode (4.1) titrate immediately and rapidly to pH 4.7 with the 0.1 N sodium hydroxide solution (3.2) by electrometry.
Carry out a blank test as follows:
Quickly place a sample of 0.2 g, weighed to the nearest mg, in a test tube with a ground glass stopper, add 10 ml of 0.1 N hydrochloric acid (3.1) and then 10 ml of urea loading agent (3.4). Cool the tube immediately in ice-water and leave there for 30 minutes. Under the conditions indicated above, transfer the contents of the tube to the titration vessel using the 0.1 N sodium hydroxide solution (3.2) up to pH 4.7.
6. Calculation of results
The urease activity is calculated by using the formula:

where:
a=ml of 0.1 N sodium hydroxide solution consumed by the sample;
b=ml of 0.1 N sodium hydroxide solution consumed in the blank test;
E=weight of the sample in grams.
7. Observations
7.1 This method is suitable for a urease activity of up to 1 mg

of N/g/minute at 30°C. For more active products, the size of the sample can be reduced to 50 mg.
7.2 Products containing more than 10% of crude fatty substance must first be defatted cold.
7.4 DETERMINATION OF VITAMIN A (RETINOL)
1. Purpose and scope
To determine the content of Vitamin A in feeding stuffs, concentrates and premixtures. The lower limit of the determination is 10,000 IU/kg for highly pigmented feeds and 4.000 IU/kg for others1. Products are classified in two groups, according to their presumed Vitamin A contents:
1 1 IU = 0.3mg of Vitamin A.
Group A: contents lower than 200,000 IU/kg;
Group B: contents equal to or greater than 200,000 IU/kg.
2. Principle
The sample is hydrolised in hot ethanolic potassium hydroxide solution and in the presence of an antioxidant or in a nitrogen atmosphere. The mixture is extracted with 1, 2-dichlorethane. The extract is evaporated to dryness and treated with light petroleum. The solution is chromatographed on a column of aluminium oxide (for Group B products, chromatography is only required in certain cases). For Group A products the Vitamin A is determined by spectrophotometry at 610 nm after development of a coloured complex according to the Carr-Price reaction; for Group B products by spectrophotometry in the UV at 325 nm.
3. Reagents
(a) used for analysing products of Groups A and B.
3.1 Ethanol, 96%(v/v).
3.2 Sodium ascorbate solution, 10% (w/v) or
3.3 Purified nitrogen.
3.4 Potassium hydroxide solution, 50% (w/v).
3.5 Potassium hydroxide solution, 1 N.
3.6 Potassium hydroxide solution. 0.5 N.
3.7 1, 2-dichlorethane.
3.8 Light petroleum, boiling range: 40-60°C; if necessary, purify as follows: stir 1,000 ml light petroleum with 20 ml lots of concentrated sulphuric acid until the acid remains colourless. Remove the acid and wash the light petroleum successively with 500 ml water, twice with 250 ml of 10% (w/v) sodium hydroxide solution and three times with 500 ml water. Remove the aqueous layer, dry the light petroleum for 1 hour over active carbon and anhydrous sodium sulphate, filter and distil.
3.9 Aluminium oxide, standardised according to Brockmann: ash for 8 hours at 750°C. cool in a desiccator and keep in a brown glass bottle fitted with a ground glass stopper. Before use in chromatography moisten as follows: place in a brown glass bottle 10 g aluminium oxide and 0.9 ml water, seal with a stopper, reheat for 5 minutes in a boiling water bath while shaking. Leave to cool. Verify the activity of the aluminium thus prepared by subjecting a known quantity of Vitamin A (3.17) (approximately 500 IU) to the procedure of 5.3 and 5.4 and checking recovery.
3.10 Basic aluminium oxide, degree of activity 1 (Woelm, Merck or equivalent).
3.11 Diethyl ether: remove peroxides and traces of water by chromatography on a column of basic aluminium oxide (3.10). (25 g

aluminium oxide per 250 ml diethyl ether).
3.12 Light petroleum solutions (3.8) containing 4, 8, 12, 16 and 20% (v/v) diethyl ether (3.11).
3.13 Sodium sulphide solution 0.5 molar in 70% (v/v) glycerine, prepared from sodium sulphide.
(b) used exclusively for analysing Group A products.
3.14 Crystallizable benzene.
3.15 Chloroform: remove the ethanol, phosgene and traces of water by chromatography on a column of basic aluminium oxide (3.10) (50 g aluminium oxide per 200 ml chloroform; it is advisable to chromatograph the first 50 ml of the eluate a second time).
3.16 Carr-Price reagent: stir approximately 25 g antimony trichloride (kept in a desiccator) with 100 ml chloroform (3.15) until the solution is saturated. A slight deposit of antimony trichloride causes no problem. Add 2 ml acetic anhydride. Keep in a refrigerator in a brown glass bottle with glass stopper. The solution keeps for several weeks.
3.17 Vitamin A — standardized spectrophotometrically.
(c) used exclusively for analysing Group B products.
3.18 Isopropanol, for chromatography.
4. Apparatus
4.1 Water bath.
4.2 Vacuum evaporation apparatus with round flasks of different capacities.
4.3 Glass chromatography tubes (length: approximately 300 mm; internal diameter: approximately 13 mm).
4.4 Spectrophotometer. Measurements in the UV require silica cells.
4.5 UV lamps suitable for 364 nm.
5. Procedure
5.1 Test sample.
From the finely divided sample, take a test sample proportional to the presumed Vitamin A content, thus:
0.1-1.0 g for concentrates (contents greater than 20,000 IU/g);
3.0-5.0 g for premixtures (contents of between 400-20,000 IU/g);
10-20 g for mineral mixtures;
30 g for Group A products.
Immediately place the test sample in a flask with a ground glass stopper.
NB. All operations must be carried out away from direct light, if necessary in brown glass equipment.
5.2 Hydrolysis and extraction1
Add successively to the test sample 40 ml ethanol (3.1), 2 ml sodium ascorbate solution (3.2)², 10 ml potassium hydroxide solution (3.4) and 2 ml sodium sulphide solution (3.13).
Heat for 30 minutes at 70-80°C under a reflux condenser and then leave to cool under a stream of water. Add 50 ml ethanol (3.1) and 100 ml 1, 2-dichlorethane (3.7). Shake vigorously and then decant the supernatant liquid into a decanting container. Add to the container 150 ml potassium hydroxide solution (3.5), shake for 30 seconds and leave to stand until the layers are separated. Collect the dichlorethane layer (lower layer) in a decanting container, add 40 ml potassium hydroxide solution (3.6), shake for 10 seconds and leave to stand until the layers are separated. Collect the dichlorethane layer in a decanting container and wash 6-8 times with 40 ml lots of water until free of alkali (phenolphthalein test).

Collect the dichlorethane layer and remove the last traces of water using strips of filter paper.
Evaporate to dryness an aliquot part of the solution under vacuum and on the water bath at 40°C. Rapidly treat the residue with 5 ml light petroleum (3.8).
For Group A products, chromatograph as shown in 5.3.1.
For Group B products, transfer the solution to a 50 ml graduated flask, make up to volume with light petroleum (3.8), mix and measure the optical density as shown in 5.4.2.
5.3 Chromatography
5.3.1 Group A products.
Fill a chromatography tube (4.3) to a height of 200 mm with aluminium oxide (3.9) previously slurried with light petroleum (3.8). Place in the tube the solution obtained in 5.2 and immediately add 20 ml light petroleum (3.8). Elute successively with 10 ml lots of the light petroleum solutions at 4, 8, 12, 16 and 20 per cent diethyl ether (3.12) under pressure or partial vacuum, the rate of flow being 2-3 drops per second.
The carotene is eluted first3. The Vitamin A is generally eluted with the light petroleum solution at 20% diethyl ether (3.12). The elution is followed under UV light (brief irradiation of the column with the mercury lamp). The fluorescent zone of the Vitamin A is clearly separated from the yellow xanthophyll zones following it. Collect the eluate fraction containing the Vitamin A in an Erlenmeyer flask.
1For milk feeds and products with a tendency to agglomerate or swell double the quantity of the reagents shown in the first and second paragraphs of 5.2.
2Sodium ascorbate need not be added when hydrolysis is carried out in a notrogen atmosphere.
3Carotene content may be determined by optical density measurement at 450 nm;

5.3.2 Group B products.
Chromatography must only be carried out if the optical density measurements obtained in 5.4.2 do not conform to the requirements given in 5.4.2.
If chromatography proves necessary, place in the chromatography column an aliquot part of the solution in the light petroleum obtained in 5.2, containing approximately 500 IU of Vitamin A, and chromatography as shown in 5.3.1.
5.4 Measurement of the optical density
5.4.1 Group A products.
Evaporate to dryness under vacuum the eluate containing the Vitamin A obtained in 5.3.1. Treat the residue with 2 ml benzene (3.14). Take 0.3 ml of this solution and add 3 ml of the Carr-Price reagent (3.16). A blue colouring develops. Measure the optical density with the spectrophotometer at 610 nm exactly 30 seconds after the reaction has begun. Determine the Vitamin A content by reference to a standard curve obtained from benzene solutions of increasing Vitamin A — standard concentrations treated with Carr-Price reagent (2-16 IU Vitamin A — standard (3.17) per 0.3 ml benzene (3.14) +3 ml Carr-Price reagent (3.16)). The standard curve must be checked regularly and frequently using the standard and a freshly prepared Carr-Price reagent solution.

5.4.2 Group B products.
Take an aliquot part of the solution in light petroleum obtained in 5.2 containing approximately 200 IU Vitamin A. Evaporate to dryness under vacuum and treat the residue with 25 ml isopropanol (3.18). Measure the optical density in the spectrophotometer at 325, 310 and 334 nm. The absorption maximum is located at 325 nm. The Vitamin A content of the solution is calculated as follows:
E32518.30=IU of Vitamin A/ml
However, the ratio of the optical densities
E310 : E325 and E334 : E325
must be 6 : 7=0.857.
If one of these ratios differs appreciably from this value (<0.830 or >>0.880), the measurement of the optical densities must be preceded by chromatography in accordance with the method given in 5.3.2. If the measurement of the optical densities carried out after chromatography shows that the above mentioned ratios still differ appreciably from the value of 0.857 (<0.830 or >>0.880), the determination must be carried out in accordance with the method given for Group A products.
6. Calculation of results
Calculate the Vitamin A content of the sample taking into account the weight of the test sample and the dilutions carried out in the course of analysis. Express the results in IU of Vitamin A per kg of feeding stuff, concentrate or premixture.
7. Repeatability
The difference between the results of two parallel determinations carried out on the same sample must not exceed:
20%, relative to the higher result, for Vitamin A contents lower than 75,000 IU/kg;
15,000 IU for contents between 75,000–150,000 IU/kg;
10%, relative to the higher result, for contents between 150,000–250,000 IU/kg;
25,000 IU for contents between 250,000–500,000 IU/kg;
5%, relative to the higher result, for contents greater than 500,000 IU/kg.
THIRD SCHEDULE
PART II
(Regulations 11 (5) and 17)
CERTIFICATE OF RESULT OF ANALYSIS

Laboratory Ref. No.
.....Sample of
.....Marked
.....received by the State Chemist on
.....from
.....
.....taken
at the premises of
.....Method
of analysis used where
two or more methods are applicable
.....This
is to certify that the above-mentioned sample, which was duly fastened and sealed, has been analysed under the direction of the State Chemist and that the result of the analysis is as

follows:—.....
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.....This certificate is given under
the European Communities (Marketing of Feedingstuffs) Regulations
1984.Date .....Signed
.....State
Chemist

FOURTH SCHEDULE

LIMITS OF ERROR REFERRED TO IN REGULATION 18(2).
1. Where, on official inspection the composition of a straight feedingstuff is found to depart from the declared composition in a manner such as to reduce its value, the following tolerances are permitted:—
(a) for crude protein:
— 2 units for declared contents of 20% or more,
— 10% of the declared content for declared contents of less than 20% but not less than 10%,
—1 unit for declared contents of less than 10%;
(b) for total sugars, reducing sugars, sucrose, lactose, glucose (dextrose) and total solids:
— 2 units for declared contents of 20% or more,
— 10% of the declared content for declared contents of less than 20% but not less than 5%.
— 0.5 unit for declared contents of less than 5%;
(c) for starch and inulin:
— 3 units for declared contents for 30% or more,
— 10% of declared content for declared contents of less than 30% but not less than 10%,
— 1 unit for declared contents of less than 10%;
(d) for crude oils and fats:

— 1.8 units for declared contents of 15% or more,
— 12% of the declared contents for declared contents of less than 15% but not less than 5%,
— 0.6 unit for declared contents of less than 5%;
(e) for moisture:
— 1 unit for declared contents of 10% or more,
— 10% of the declared content for declared contents of less than 10% but not less than 5%,
— 0.5 unit for declared contents of less than 5%;
(f) for total phosphorus, sodium, calcium carbonate, calcium, magnesium, acid index and matter insoluble in light petroleum:
— 1.5 units for declared contents (values) of 15% (15) or more, as appropriate,
— 10% of the declared content (value) for declared contents (values) of less than 15% (15) but not less than 2%(2), as appropriate;
— 0.2 unit for declared contents (values) of less than 2% (2) as appropriate;
(g) for ash insoluble in hydrochloric acid and chlorides expressed as NaCl:
— 10% of the declared content for declared contents of 3% or more,
— 0.3 unit for declared contents of less than 3%;
(h) for carotene, vitamin A and xanthophyll:
— 30% of the declared content;
(i) for methionine, cystine, lysine and volatile nitrogenous bases:
— 20% of the declared content;
(j) for crude fibre:
—2.2 units for declared contents of 11% or more,
— 20% of the declared content for declared contents of less than 11% but not less than 7%,
— 1.4 units for declared contents of less than 7%,
(k) for crude ash:
— 1.5 units for declared contents of 10% or more,
— 15% of the declared content for declared contents of less than 10% but not less than 4%,
— 0.6 unit for declared contents of less than 4%;
(l) for Hectolitre Weight:
—1.5 units.
(m) for Nitrogen:
— 0.4 unit.
2. Where, on official inspection the composition of a compound feedingstuff other than for dogs and cats is found to depart from the declared composition, the following tolerances are permitted:—
(i) Where the content recorded is less than the declared content:
(a) Crude protein and skim milk powder:
— 2 units for declared contents of 20% or more,
— 10% of the declared content for declared contents of less than 20% but not less than 10%,
— 1 unit for declared contents of less than 10%;
(b) Soluble protein:
— 3 units for declared contents of 25% or more,
— 12% of the declared content for declared contents of less than 25% but not less than 15%,
— 1.8 units for declared contents of less than 15%;
(c) Total sugar:
— 2 units for declared contents of 20% or more,

—10% of the declared content for declared contents of less than 20% but not less than 10%,
— 1 unit for declared content of less than 10%;
(d) Starch and total sugar plus starch:
— 2.5 units for declared contents of 25% or more,
— 10% of the declared content for declared contents of less than 25% but not less than 10%,
— 1 unit for declared contents of less than 10%;
(e) Crude oils and fats:
— 1.5 units for declared contents of 15% or more,
— 10% of the declared content for declared contents of less than 15% but not less than 8%,
— 0.8 unit for declared contents of less than 8%;
(f) Sodium and magnesium:
— 1.5 units for declared contents of 15% or more,
— 10% of the declared content for declared contents of less than 15% but not less than 7.5%,
— 0.75 unit for declared contents of less than 7.5% but not less than 5%,
— 15% of the declared content for declared contents of less than 5% but not less than 0.7%,
— 0.1 unit for declared contents of less than 0.7%;
(g) Total phosphorus and calcium:
— 1.2 units for declared contents of 16% or more,
—7.5% of the declared content for declared contents of less than 16% but not less than 12%,
— 0.9 unit for declared contents of less than 12% but not less than 6%,
— 15% of the declared content for declared contents of less than 6% but not less than 1%,
— 0.15 unit for declared contents of less than 1%;
(h) Methionine and lysine:
—15% of the declared content;
(i) Cystine:
— 20% of the declared content.
(ii) Where the content recorded is more than the declared content:
(a) Moisture:
— 1 unit for declared contents of 10% or more,
— 10% of the declared content for declared contents of less than 10% but not less than 5%,
— 0.5 unit for declared contents of less than 5%;
(b) Crude ash:
— 1.5 units for declared contents of 10% or more,
— 15% of the declared content for declared contents of less than 10% but not less than 4%,
— 0.6 unit for declared contents of less than 4%;
(c) Crude fibre:
— 2.2 units for declared contents of 11% or more,
— 20% of the declared content for declared contents of less than 11% but not less than 7%,
— 1.4 units for declared contents of less than 7%;
(d) Ash insoluble in hydrochloric acid:
— 1 unit for declared contents of 10% or more,
— 10% of the declared content for declared contents of less than 10% but not less than 4%,

— 0.4 unit for declared contents of less than 4%;
(iii) Where the variation noted is in the opposite direction to those referred to respectively in paragraphs 2 (i) and 2 (ii):
(a) — crude protein, crude oils and fats, total sugar, starch: tolerance twice that permitted for these substances in paragraph 2 (i);
(b) — total phosphorus, calcium, magnesium, sodium, crude ash, crude fibre; tolerance three times that permitted for these substances in paragraphs 2 (i) and 2 (ii).
3. Where, on official inspection the composition of a compound feedingstuff for dogs and cats is found to depart from the declared composition, the following tolerances are permitted:
(i) Where the content recorded is less than the declared content;
(a) Crude protein:
— 3.2 units for declared contents of 20% or more;
— 16% of the declared content for declared contents of less than 20% but not less than 12.5%;
— two units for declared contents of less than 12.5%.
(b) Crude oils and fats:
— 2.5 units of the declared content.
(ii) Where the content recorded is more than the declared content:
(a) Moisture:
— 3.0 units for declared contents of 40% or more,
— 7.5% of the declared content for declared contents of less than 40% but not less than 20%,
— 1.5 units for declared contents of less than 20%.
(b) Crude ash:
— 1.5 units of the declared content.
(c) Crude Fibre:
— 1.0 unit of the declared content.
(iii) Where the variation noted is in the opposite direction to those referred to respectively in paragraphs 3 (i) and 3 (ii):
(a) Crude protein:
— tolerance twice that permitted for this substance in paragraph 3 (i).
(b) Crude oils and fats:
— identical tolerance,
(c) Crude ash, crude fibre:
— tolerance three times that permitted for these substances in paragraph 3 (ii).
GIVEN under my Official Seal, this 31st day of July, 1984.

AUSTIN DEASY,
Minister for Agriculture.

EXPLANATORY NOTE.
The Regulations which cover the marketing of all feedingstuffs other than specified exceptions, lay down quality, labelling and packing requirements for straight and compound feedingstuffs, as well as official methods of sampling and analysis. They revoke the Fertilisers, Feeding Stuffs and Mineral Mixtures Regulations 1957 (S.I. No. 264 of 1957) other than Regulation 13, insofar as they relate to feedingstuffs and mineral mixtures.