A.L. 99 ta' 2005

ATT DWAR IS-SERVIZZI VETERINARJI (KAP. 437)

Regoli ta' l-2005 dwar Analiżi għall-Kontroll Uffiċjali tal-Għalf, 2004

BIS-SETGĦA mogħtija taħt artiklu 22 ta' l-Att dwar is-Servizzi Veterinarji, il-Ministru ta' l-Ambjent u l-Affarijiet Rurali għamel dawn ir-Regoli:-

Titlu, skop u applikabilità.

1. (1) It-titlu ta' dawn ir-regoli hu Regoli għall-Analiżi għall-Kontroll Uffiċjali ta' l-Għalf.

(2) L-iskop ta' dawn ir-Regoli hu biex jiġu implimentati ir-regoli taħt id-Direttiva tal-Kummisssjoni ta' l-UE 71/393/KEE li titstabbilixxi metodi għall-analiżi għall-kontroll uffiċjali ta' l-għalf.

(3) L-analiżi għall-kontroll uffiċjali ta' l-għalf li għandu kontenut ta' ilma, nitroġeni volatili, żjut fosforiċi u mhux maħdumin totali, u xaħam, għandhom isiru skond il-metodi li hemm fl-Iskeda ta' dawn ir-regoli.

SKEDA

1. DETERMINAZZJONI TA' L-ILMA

1. **Ghan u Skop**

Dan il-metodu jiddetermina l-kontenut ta' l-ilma fl-ghalf. Ma jkoprix l-analizi tal-prodotti tal-halib bhala ghalf wahdu, l-analizi tas-sustanzi minerali u tahlit compost principalment minn sustanzi minerali, l-analizi ta' xahmijiet u zjut kemm mill-annimali u kemm mil-haxix jew l-analizi taz-zerriegha u taz-zejt u frott zejtni mnizzlin fir-Regolament tal-Kunsill ta' l-UE 136/66/KEE tat-22 ta' Settembru 1966 dwar organizzazzjoni komuni taz-zejt u x-xaham fis-suq.

Id-determinazzjoni tal-kontenut ta' ilma fiz-zerriegha taz-zejt u frott zejtni hi mnizzla fit-tielet Anness tar-Regolament tal-Kummissjoni tal-UE [illegible] 1470/68 tat-23 ta' Settembru 1968 dwar id-*drawing* u r-riduzzjoni ta' kampjuni u d-determinazzjoni tal-kontenut taz-zejt, impuritajiet u ilma fiz-zerriegha taz-zejt.

2. **Principju**

Il-kampjun jigi mnixxef f'kundizzjonijiet specifici li jverjaw skond in-natura ta'l-ghalf. It-telf fil-massa huwa mkejjel billi tiznu. Hu necessarju li jsir tnixxif preliminary fil-kaz ta' ghalf solidu li ghandu kontenut gholi ta' ilma.

3. **Apparat**

3.1 *Crusher* maghmul minn materjal li ma jassorbix ilma li jitnaddaf facilment u jhalli l-*crushing* minghajr ma jkun hemm hafna shana, ma jhallix kemm jista' jkun kuntatt ma' l-arja ta' barra u jissodisfa r-rekwiziti mnizzla f' 4.1.1 u 4.1.2 (ez *hammer* jew *water-cooled micro-crushers, collapsible cone mills, slow motion* jew *cogwheeled crushers.)*

3.2 Bilanc analitiku*;* sa l-eqreb 0.5mg.

3.3 Kontenituri nixfin ta' metal li ma jikkorrodix ruhu jew tal-hgieg b' ghatu li jassikura li jinghalqu u ma tidholx arja; superfici fejn isir ix-xoghol li jippermetti l-kampjun tat-test biex jinfirex xi 0.3g/cm kwadri.

3.4 Forn isotermiku li huwa msahhan bl-elettriku (+/- 1 grad Celsius) li jkun regolat sew u li jizgura regolazzjoni tat-temperatura rapida [3].

3.5 Forn tal-valur aggustabbli li jissahhan bl-elettriku b'pompa taz-zejt u jew mekkanizmu biex tiddahhal arja shuna u niexfa jew agent ta' tnixxif (ez *calcium oxide*) .

3.6 Dessikatur bi *plate* hoxna u perforata tal-metall jew porcellana , li fiha agent ta' tnixxif efficjenti.

4. **Procedura**

N.B: L-operazzjonijiet imnizzla f' din is-sezzjoni jriidu jsiru immedjatament wara li jinfetħu l-pakketti tal-kampjuni. L-analizi trid issir mill-inqas darbtejn.

4.1 *Preparazzjoni*

4.1.1 *Ghalf hlief dak li jaqa' taht 4.1.2 u 4.1.3*

Hu mill-inqas 50g mill-kampjun . Jekk ikun necessarju, farrak jew aqsam f'mod li tigi evitata kull varjazzjoni fil-kontenut tal-ilma. (ara 6)

4.1.2 *Cereali u groats*

Hu mill-inqas 50g mil-kampjun. Farrak f'bicciet li minnhom mill-inqas 50% jista' jghaddi minn xibka ta' 0.5mm u thalli mhux iktar minn 10% *reject* fuq xibka tal-toqob tondi tal-1mm.

4.1.3 *Ghalf f'forma ta' likwidu jew paste, ghalf li ghandu kontenut ewlieni ta' zejt u xaham*

Hu xi 25g mil-kampjun, izen sa l-eqreb 10mg, zid ammont xieraq ta' ramel anidriku mwiezen sa l-eqreb 10mg u hallat sakemm ikollok tahlita omo genika.

4.2 *Tnixxif*

4.2.1 *Ghalf li ma jikklassifikax taht 4.2.2 u 4.2.3*

Izen kontenitur (3.3) bl-ghatu sa l-eqreb 0.5mg. Poggi fil-konenitur, sa l-eqreb 1mg, xi 5g mil-kampjun u xerred sew. Poggi l-kontenitur, minghajr l-ghatu, fil-forn li jkun issahhan minn qabel sa 103 gradi Celsius. Biex it-temperatura tal-forn ma tinzilx malajr, dahhal il-kontenitur malajr kemm jistra' jkun. Hallih jinxef ghal erba' sighat ikkalkulati minn meta t-temperatura terga' taqa' sa 103 gradi Celsius. Erga poggi l-ghatu fuq il-kontenitur, ohrog il-kontenitur mil-forn, hallih

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jiehu jitnixxfu ghal saghtejn, ir-rizultati jvarjaw minn dawk ta' erba' sighat qabel b'inqas minn 0.15%.

jiksah bejn 30 u 45-il minuta fid-dessikatur (3.6) u izen sa l-eqreb 1mg.

Fil-kaz ta' ghalf komposti principalment minn zejt u xaham, nixxef ghal 30 minuta ohra f' temperatura ta' 130 grad Celsius. Id-differenza bejn uzin u iehor ma tistax teccedi 0.1% ilma.

4.2.2 *Dqiq, groats u meal*

Izen kontenitur (3.3) bl-ghatu sa l-eqreb 0.5mg. Poggi fil-konenitur, sa l-eqreb 1mg, xi 5g mil-kampjun u xerred sew. Poggi l-kontenitur,minghajr l-ghatu, fil-forn li jkun issahhan minn qabel sa 103 gradi Celsius. Biex it-temperatura tal-forn ma tinzilx malajr, dahhal il-kontenitur malajr kemm jistra' jkun. Hallih jinxef ghal saghtejn ikkalkulati minn meta t-temperatura terga' taqa' sa 103 gradi Celsius. Erga poggi l-ghatu fuq il-kontenitur, ohrog il-kontenitur mil-forn, hallih jiksah bejn 30 u 45-il minuta fid-dessikatur (3.6) u izen sa l-eqreb 1mg.

4.2. *Ghalf kompost li fih iktar minn 4% sukrozju jew laktozju: ghalf wahdu bhal locust beans,prodotti tac-cereali idrolizzati, zerriegha tal-malt, dried beet chips, solubbli tal-hut u z-zokkor; ghalf kompost li fih iktar minn 25% minerali inkluz ilma tal-kristallizzazzjoni.*

Izen kontenitur (3.3) bl-ghatu sa l-eqreb 0.5mg. Poggi fil-kontenitur, sa l-eqreb 1mg, xi 5g mil-kampjun u xerred sew. Poggi l-kontenitur, minghajr l-ghatu, fil-forn tal-vakum li jkun issahhan minn qabel bejn 80 u 85 grad celsius. Biex it-temperatura tal-forn ma tinzilx malajr, dahhal il-kontenitur malajr kemm jistra' jkun.

Tella' l-pressjoni sa 100 Torr u hallih jinxef ghal erba' sighat f'din il-pressjoni , jew f'kurrent ta' arja shuna u niexfa jew permezz ta' agent li jnixxef (xi 200g ghal 20 kampjun). Fis-sitwazzjoni ta' l-ahhar aqla' l-*vacuum pump* meta tintlahaq il-pressjoni mehtiega. Ikkalkula l-perijodu ta' tnixxif minn meta t-temperatura tal-forn tinzel ghal 80 sa 85 grad Celsius. Erga gib il-pressjoni tal-forn attentament ghal-pressjoni ambjentali. Ifteh il-forn, poggi l-ghatu fuq il-kontenitur, ohrog il-kontenitur mil-forn, hallih jiksah bejn 30 u 45-il minuta fid-dessikatur (3.6) u erga' izen. Id-differenza bejn uzin u iehor ma tistax teccedi 0.1% ilma.

4.3 Tnixxif preliminari

4.3.1 *Ghalf li ma jikklassifikax taht 4.3.2*

Ghalf solid b'kontenut gholi ta' ilma li jikkawza diffikulta' fil-tifrik tal-kampjun irid jkun soggett ghal tnixxif preliminari kif gej:

Izen 50 g mil-kampjun mhux imfarrak sa l-eqreb 10mg (Ghalf kompressat jew agglomerat jista' jitqassam approssimament jekk ikun necessarju) f'kontenitur xieraq (ez *aluminium plate* ta' 20 * 12cm b' rim ta' 0.5cm). Hallih jinxef f' forn bejn 60 u 70 grad Celsius sakemm l-ilma li hemm fih jitnaqqas sa bejn 8% u 12%. Ohorgu mil-forn, hallih jiksah minghajr ghatu fil-laboratorju ghal siegha u iznu sa l-eqreb 10mg. Farrku mil-ewwel kif indikat f' 4.1.1 u nixfu kif indikat f' 4.2.1 jew 4.2.3 skond in-natura ta' l-ghalf.

4.3.2 *Cereali*

Qamh b'kontenut ta' ilma ta' iktar minn 17% jrid ikun soggett ghal tnixxif preliminari kif gej:

Izen 50 g mil-qamh mhux imfarrak sa l-eqreb 10mg (Ghalf ikkompressati jew agglomerate jista' jitqassam approssimament jekk ikun necessarju) f'kontenitur xieraq (ez *aluminium plate* ta' 20 * 12cm b' rim ta' 0.5cm). Hallih jinxef f' forn bejn 60 u 70 grad Celsius ghal 5 sa 7 minuti . Ohorgu mil-forn, hallih jiksah minghajr ghatu fil-laboratorju ghal saghtejn u iznu sa l-eqreb 10mg. Farrku mil-ewwel kif indikat f' 4.1.2 u nixfu kif indikat f' 4.2.2 skond in-natura tal-*feedingstuff*.

5. **Kalkolu tar-rizultati**

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

Tnixxif wara li jkun diga` gie mnixxef

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

Fejn:

E= massa inizjali, fi grammi, ta' l-ezami tal-kampjun.
M= massa, fi grammi, ta' l-ezami tal-kampjuni wara t-tnixxif preliminari.
M'= massa, fi grammi, ta' l-ezami tal-kampjuni wara li jkun imfarrak u midhun.
M = massa, fi grammi, ta' l-ezami niexef tal-kampjuni.

5.3 *Repetizzjoni*

Id-differenza bejn ir-rizultati ta' zewg determinazzjonijiet paralleli mill-istess kampjun ma jistghux jeccedu 0.2% ilma.

6. **Osservazzjoni**

Jekk it-tifrik ikun necessarju u jekk dan jaltera l-kontenut ta' ilma tal-prodott, ir-rizultati ta'l-analizi tal-komponenti ta'l-ghalf iridu jitrangaw fuq il-bazi tal-kontenut tal-ilma tal-kampjun fl-istat inizzjali tieghu.

II **DETERMINAZZJONI TA' BAZIJIET NITROGENI VOLATILI**

A. MIKRODIFFUZJONI

1. **Ghan u skop**

Dan il-metodu jippermetti d-determinazzjoni tal-kontenut ta' bazijiet nitrogeni volatili, espressi bhala *ammonia*, fl-ghalf.

2. Principju

Il-kampjun jigi estratt bl-ilma u s-soluzzjoni tigi iccarata u iffiltrata. Il-bazijiet nitrogeni volatili jigu spustati bil-mokrodiffuzjoni permezz ta' soluzzjoni ta' *potassium carbonate* , migbura f' soluzzjoni ta' *boric acid* u titrati bis-*sulphuric acid*.

3. **Reagenti**

3.1. 20% (w/v) soluzzjoni ta' *trichloroacetic acid*.

3.2. Indikatur: holl 33 mg ta' *bromocresol green* u 65 mg *methyl red* f' 100 ml 95%—96% (v/v) *ethanol*.

3.3. Soluzzjoni ta' *boric acid:* holl fi *graduated flask* tal-1 litru 10 g *boric acid A.R* f' 200 ml ta' 95%—96% (v/v) *ethanol* u 700 ml ilma. Zid 10 ml indikatur (3.2.). Hallat u, jekk ikun necessarju, adatta l-kulur tas-soluzzjoni ghal ahmar car billi zzid soluzzjoni tas-*sodium hydroxide*. 1 ml minn din is-soluzzjoni tahdem fuq massimu ta' 300 µg NH_3.

3.4. Soluzzjoni ta' *potassium carbonate* issaturata : Holl 10.0g *potassium carbonate A.R.* f' 100 ml ilma jaghli. Hallih jiksah, u iffiltrah.

3.5. *Sulphuric acid* 0·02 N.

4. **Apparat**

4.1. *Mixer (tumbler*): approssimament bejn 35 u 40 r.p.m.

4.2. *Conway cells* tal-hgieg jew tal-plastik (ara d-*diagram*).

4.3. *Microburettes* iggradwati f' 1/100 ml.

5. **Procedura**

Izen 10g mil-kampjun sa l-eqreb 1mg u poggih f' 100ml ilma f' *graduated flask* tal-200ml. Hallat fit-*tumbler* ghal 30 minuta. Zid 50ml soluzzjoni ta' *trichloroacetic acid* (3.1), zid sal-marka bl-ilma, ixxekja bil-qawwa u iffiltra b'*pleated filter*.

Permezz ta' pipetta, qattar 1ml soluzzjoni ta' *boric acid* (3.3) fil-parti centrali tal-*Conway cell* u 1ml mil-filtrat tal-kampjun fil-*crown* tac-cellula. Aghtti parti minnha bl-ghatu iggrisjat. Qattar 1ml soluzzjoni issaturata ta' *potassium carbonate*(3.4) malajr fil-*crown* u aghlaq l-ghatu halli c-cellula tkun izolata ghal kollox mill-arja. Dawwar ic-cellula attentament u poggiha orizzontalmet biex iz-zewg reagenti jithalltu. Halliha inkubata ghal mill-inqas erba' sighat f'temperatura ambjentali jew ghal siegha f'40 grad Celsius.

Permezz ta' *microburette (4.3)*, ittitratja il-bazijiet volatili fis-soluzzjoni ta' *boric acid* bis-*sulphuric acid* 0.02 N (3.5).

Aghmel *blank test* bl-istess procedura izda minghajr kampjun biex jigi analaizzat.

6. **Kalkolu tar-Rizultati**

1ml H2SO4 0.02N jikkorrispondu ghal 0.34mg *ammonia*.

Esprimi r-riziltat bhala persentagg tal-kampjun.

Repetizzjoni

Id-differenza bejn ir-rizultati ta' zewg determinazzjonijiet paralleli mill-istess kampjun ma jistghux jeccedu :

10%, valur relattiv, ghal kontenut ta'*ammonia* ta' inqas minn 1%;

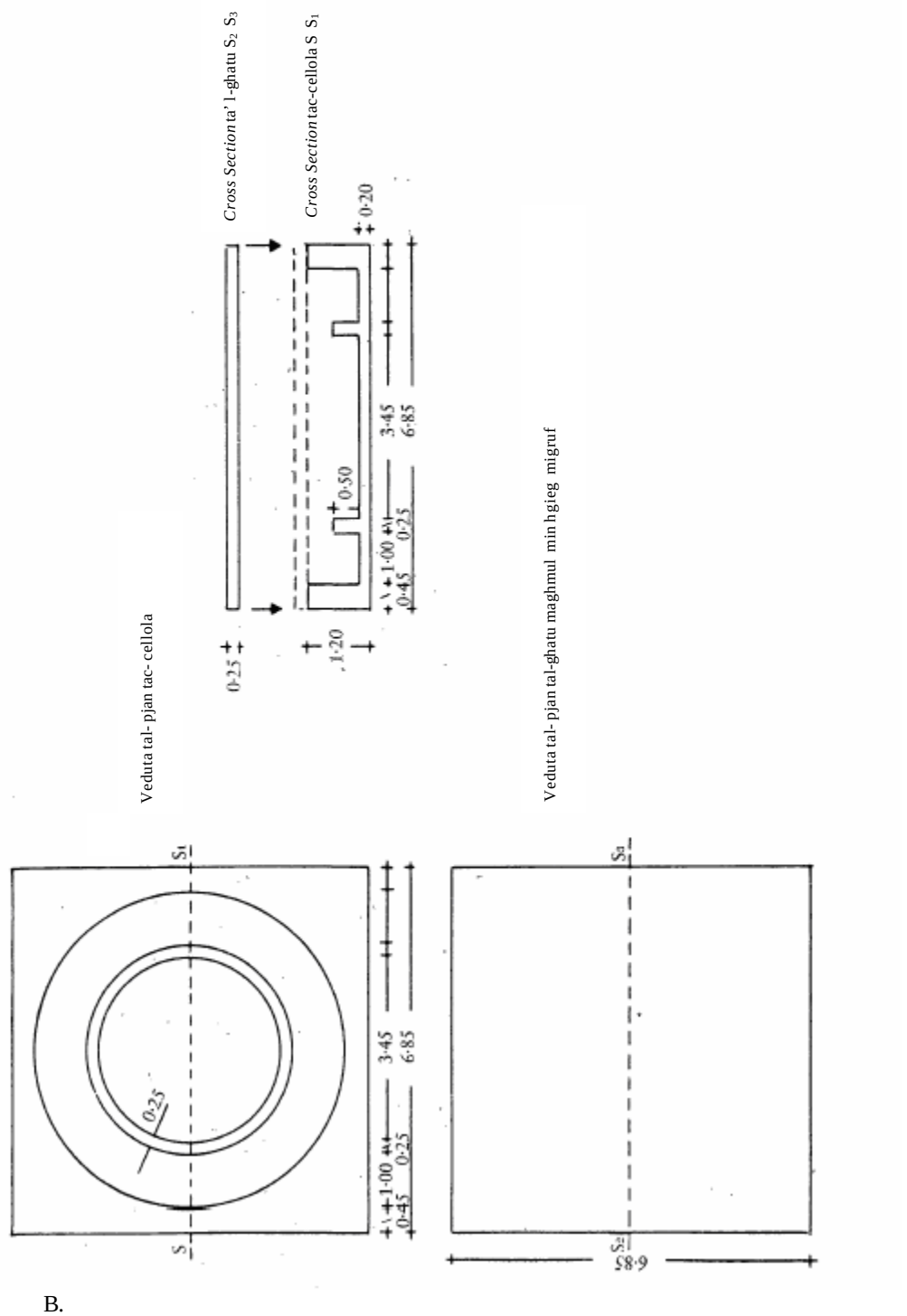
0.1%, valur assolut, ghal kontenut ta' *ammonia* ta' 1.0% jew iktar.

7. **Osservazzjoni**

Jekk il-kontenut ta' l-*ammonia* jeccedi 0.6%, hallat il-filtrat inizzjali.

Cellola ta' *Conway*

Skala 1/1



B.

BID-DISTILLAZZJONI

1. **Ghan u skop**

Dan il-metodu jippermetti d-determinazzjoni tal-kontenut ta' bazijiet nitrogeni volatili, espress bhala *ammonia,* fil-*fish meal* li ma fih prattikalment ebda *urea.* Dan il-metodu japplika biss fil-kaz ta' kontenut ta' *ammonia* ta' inqas minn 0.25%.

2. **Principju**

Il-kampjun jigi estratt bl-ilma u s-soluzzjoni iccarata u iffiltrata . Il-bazijiet nitrogeni volatili jigu spustati fil-*boiling point* billi zzid il-*magnesium oxide* u tigbru f' ammont specifiku ta' *sulphuric acid*, u l-eccess tieghu jigi titrati lura f' soluzzjoni ta' *sodium hydroxide.*

Reagenti

3.1. 20% (w/v) soluzzjoni *trichloroacetic acid*.

3.2. *Magnesium oxide A.R.*

3.3. *Anti-foaming emulsion* (ez. *silicone*).

3.4. *Sulphuric acid* 0·1 N.

3.5. Soluzzjoni ta' s*odium hydroxide* 0·1 N.

3.6. 0·3% (w/v) soluzzjoni ta' *methyl red* f' 95%—96% (v/v) *ethanol*.

4. **Apparatus**

4.1. *Mixer (tumbler)*: approssimament 35 sa 40 r.p.m.

4.2. Apparat ghad-distillazzjoni tat-tip *Kjeldahl*.

5. **Procedura**

Izen 10g mil-kampjun sa l-eqreb 1mg u poggi ma' 100ml ilma f'*graduated flask tal-200ml.* Hallat fit-*tumbler* ghal 30 minuta. Zid 50ml soluzzjoni ta' *trichloroacetic acid* (3.1), zid sal-marka bl-ilma, ixxekja bil-qawwa u iffiltra permezz ta' *pleated filter*.

Hu kemm hemm bzonn mil-filtrat car ghal-kontenut mistenni tal-bazijiet nitrogeni volatili (100ml ikunu bizzejjed is-soltu). Hallat sa 200ml u zid 2g *magnesium oxide* (3.2) u ftit qtar ta' *anti-foaming emulsion* (3.3). Is-soluzzjoni trid tkun *alkaline* fuq il-*litmus paper* , jekk le zid ftit *magnesium oxide (3.2).* Iddistilla xi 150ml mis-soluzzjoni fil-*Kjeldahl apparatus* u igbor id-distillat f'*Erlenmeyer flask* li fih volum preciz ta' (25 sa 50ml) *sulphuric acid* 0.1N (3.4). Waqti d-distillazzjoni, evita l-*overheating* tal-gnub. Aghlli s-*sulphuric acid* ghal zewg

minuti, keshu u aghmel *back-titration* ta' l-eccess ta' *sulphuric acid* bis-soluzzjoni ta' *sodium hydroxide* 0.1N (3.5) fil-prezenza indikatur tal-*methyl red (3.6).*

Aghmel *blank test* bl-istess procedura izda minghajr kampjun ghall-analizi.

6. **Kalkulazzjoni tar-Rizultati**

1ml H2SO4 0.1N jikkorrispondi ghal 1.7mg *ammonia.*

Esprimi r-rizultat bhala persentagg tal-kampjun.

Repeatiblitiy

Id-differenza bejn ir-rizultati ta' zewg determinazzjonijiet paralleli mill-istess kampjun ma ghandhomx jeccedu 10%, valur relattiv ta' *ammonia.*

III. **DETERMINAZZJONI TAT-FOSFRU TOTALI**

METODU FOTOMETRIKU

1. **Ghan u sskop**

Dan il-metodu jiddetermina l-kontenut ta' fosfru totali fl-ghalf. Huwa partikolarment approprjat ghall-analizi tal-prodotti b'kontenut baxx ta' fosfru. F' certu kazijiet (prodotti li fihom hafna fosfru), jista' jintuza metodu gravimetriku.

2. **Principju**

Il-kampjun jigi immineralizzat, jew bid-kombustjoni niexfa (fil-kaz ta' ghalf organici) inkella bl-digestjoni acidika (fil-kaz ta' kompostiminerali u ghalf likwidu), u mqieghdin f'soluzzjoni aciduza. Is-soluzzjoni tigi trattata bil-*molybdovanadate reagent*. L-densita` ottika tas-soluzzjoni safra li tifforma titkejjel f' spektrofotometru f' 430nm.

3. **Reagents**

3.1. *Calcium carbonate A.R.*

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

3.3. *Nitric acid A.R.*, d: 1·045.

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

3.5. *Sulphuric acid A.R.,* d: 1·84.

3.6. *Molybdovanadate reagent*: hallat 200 ml soluzzjoni ta' *ammonium heptamolybdate* (3.6.1), 200 ml soluzzjoni ta' *ammonium monovanadate* (3.6.2) u 134 ml *nitric acid* (3.4) f' *graduated flask* tal-I litru. Imla sal-marka bl-ilma.

3.6.1. Soluzzzjoni ta' *ammonium heptamolybdate* : holl 100 g *ammonium heptamolybdate A.R.* (NH4) 6Mo7O24.4H2O. Zid 10ml *ammonia* (d: 0·91) u imla sal-marka ta' 1litru bl-ilma.

3.6.2. Soluzzjoni ta' *ammonium monovanadate*: Holl 2·35 g *ammonium monovanadate* A.R. NH4VO3 f' 400 ml ilma jahraq. Filwaqt li thawwad bla waqfien, zid bil-mod 20 ml *dilute nitric acid* dilwit (7 ml HNO3 (3.4) + 13 ml H2O) u imla sal-marka ta' 1 litru bl-ilma..

3.7. *Standard solution* ta' 1 mg fosfru ghal kull ml: holl 4·387 g *potassium dihydrogen phosphate* A.R. KG2PO4 fl-ilma. Imla sal-marka ta' 1 litru bl-ilma.

4. **Apparat**

4.1. *Silica* jew *porcelain ashing crucibles.*

4.2 *Muffle-furnace* ta' l-elettriku bit- termostat issetjat fuq 550 ºC.

4.3. 250 ml *Kjeldahl flask*.

4.4. *Graduated flasks* u pipette tal-precizjoni.

4.5. Spektrofotometru.

4.6. *Test tubes* b'diametru ta' xi 16mm, b' *stoppers* iggradati ghal diametru ta' 14·5 mm; kapacita': 25 sa 30 ml.

5. **Procedura**

5.1 *Preparazzjoni tas-soluzzjoni*

Skond in-natura tal-kampjun , ipprepara s-soluzzjoni kif indikat f' 5.1.1 jew 5.1.2.

5.1.1 *Procedura tas-soltu*

Izen 1g jew iktar mil-kampjun sa l-eqreb img. Poggi l-kampjun tat-test f'*Kjeldahl flask*, zid 20ml *sulphuric acid(3.5)*, ixxekja biex timla s-sustanza kompletament bl-acidu u biex tevita li tehel mal-gnub tal-*flask*, sahhan u zomm fuq il-*boiling point* ghal ghaxar minuti. Halliha tiksah ftit, zid 2ml *nitric acid* (3.4), sahhan ftit, halliha tiksah ftit, zid ftit iktar *nitric acid* (3.4) u erga' gibha ghal-*boiling point*. Irrepeti l-procedura sakemm ikollok soluzzjoni bla kulur. Kessah, zid ftit ilma, iddekantja l-likwidu f'*graduated flask* tal-500ml, u lahlah il-*Kjeldahl flask* bl-ilma

jahraq. Hallih jiksah, imla sal-marka bl-ilma, omogenizza u iffiltra.

5.1.2 Kampjuni li fihom sustanzi organici u minghajr *calcium* u *magnesium dihydrogen phosphates*

Izen xi 2.5g mil-kampjun sa l-eqreb mg f' *ashing crucible*. Hallat il-kampjun tat-test sakemm ikun imhallat kompletament ma' 1g *calcium carbonate* (3.1). Axxjah fil-forn f'temepratura ta' 550 grad ceslsius +/- 5 gradi Celsius sakemm ikollok irmied abjad jew griz (ma jimpurtax jekk ikun hemm xi ftit *charcoal*)

Poggi l-irmied f' *beaker* tal-250ml . Zid 20ml ilma u *hydrochloric acid (3.2)* sakemm tieqaf l-effervexxenza . Zid 10ml ohra *hydrochloric acid* (3.2). Poggi l-*beaker* fuq *sand bath* u evapora sakemm jinxef biex is-*silica* tkun insolubli. Erga' holl ir-residwu f'10ml *nitric acid* (3.3) u aghllih fuq is-*sand bath* ghal 5 minuti minghajr ma tevapora sakemm jinxef. Iddekantja l-likwidu go *graduated flask* tal-500ml, u lahlah il-*beaker* ghal numru ta' darbiet bl-ilma jahraq. Hallih jiksah, zid sal-marka bl-ilma, onogenizzah u iffiltrah.

5.2 Zvilupp tal-kulur u tkejjil tad-densita` ottika

Hallat parti alikwota tal-filtrat minn 5.1.1 jew 5.1.2 biex ikollok koncentrazzjoni ta' *phosphorus* ta' mhux iktar minn 40 ug/ml. Poggi 10ml minn din is-soluzzjoni f' *test tube* (4.6) u zid 10ml *molybdovanadate reagent* (3.6). Omogenizzah u hallih joqghod ghal mill-inqas 10 minuti f' temepratura ta' 20 grad Celsius. Kejjel id-densita` ottika f'spektrofotometru fuq 430nm kontra soluzzjoni billi zzid 10ml *molybdovanadate reagent* (3.6) ma' 10ml ilma.

5.3 Kurva ta' Kalibrazzjoni

Mis-*standard solution* (3.7) ipprepara soluzzjonijiet b' 5, 10, 20, 30, 40 u 50 ug *phosphorus* ghal kull ml rispettivament. Hu 10ml minn kull soluzzjoni u zid 10ml *molybdovanadate reagent* (3.6). Omogenizzah u hallih joqghod ghal mill-inqas 10 minuti f' temperatura ta' 20 grad Celsius. Kejjel id-densita` ottika kif indikat f'5.2.

Ittrejsja l-*calibration curve* billi tipplottja id-densitajiet ottici kontra l-kwantitajiet korrispondenti ta' fosfru. Ghal koncentrazzjonijiet bejn 0 u 40ug/ml, il-kurva ghandha tkun lineari.

6. **Kalkolu tar-rizultati**

Iddetermina l-ammont ta' fosfru fil-kampjun tat-test permezz tal-kurva ta' kalibrazzjoni.

Esprimi r-riziltat bhala persentagg tal-kampjun.

Repetizzjoni

Id-differenza bejn ir-rizultati ta' zewg determinazzjonijiet paralleli mill-istess kampjun ma jistghux jeccedu :

3%, valur relattiv, ghal kontenut ta'fosfru ta' inqas minn 5%;

0.15%, valur assolut, ghal kontenut ta' fosfru ta' 5% jew iktar.

IV. **DETERMINAZZJONI TA' ZJUT MHUX MAHDUMIN U XAHMIJIET**

1. **Ghan u skop**

Dan il-metodu jiddetermina l-zjut mhux mahdumin u l-xahmijiet fl-ghalf. Ma jkoprix l-analizi taz-zerriegha taz-zejt u frott zejtni iddefinit fir-Regolament tal-Kunsill tal-Unjoni Ewropea 136/66/KEE tat-22 ta' Settembru 1966.

L-uzu taz-zewg proceduri deskritti hawn taht jiddependi mil-kompozizzjoni u u n-natura ta' l-ghalf u r-raguni ghall-analizi.

1.1 *Procedura A – zjut mhux mahdumin u xahmijiet li jistghu jigu estratti direttament*

Dan il-metodu japplika ghal hwejjeg ta' l-ikel li gejjin mil-pjanti, hlief dawk li jaqghu taht Procedura B.

1.2 *Procedura B – zjut mhux mahdumin u xahmijiet totali*

Dan il-metodu japplika ghal hwejjeg ta' l-ikel li gejjin mill-annimali u ghal- ikel kompost. Ghandu jintuza ghal kull materjal ta' l-ikel li minnu ma jistghux jigu kompletamant estratti zjut u xaham minghajr *hydrolysis* (ez *glutens,* hmira, proteina tal-patata u prodotti soggetti ghal processi bhal *extrusion, flaking u* tishin*).*

1.3 *Interpretazzjoni tar-rizultati*
F'kull kaz fejn ikollok rizultat permezz ta' Procedura B li jkun oghla minn dak li ta' Procedura A, ir-Rizultat ta' Procedura B ghangu jkun accettat bhala l-valur reali.

2. **Principju**

2.1 *Procedura A*

Il-kampjun jigi estratt bil-*light petroleum.* Is-solvent jitnehha bid-distillazzjoni u r-residwu mnixxef u mwiezen.

2.2 *Procedura B*

Il-kampjun jigi trattat bit-tishin bil-*hydrochloric acid*. It-tahlita trid titkessah u tigi filtrata. Ir-residwu jrid jinhasel u jitnixxef u iddeterminat skond il-Procedura A.

3. **Reagents**

3.1. *Light petroleum, boiling range*: bejn 40 u 60 ºC. Il-valur tal- *bromine* irid ikun inqas minn 1 u r-residwu ta' l-evaporazzjoni inqas minn 2 mg/100 ml.

3.2. *Sodium sulfate, anhydrous*.

3.3. *Hydrochloric acid*, c = 3 mol HCl/l

3.4. Ghajnuna ghall-filtrazzjoni ez. *Kieselguhr, Hyflo-supercel*.

4. **Apparat**

4.1. Apparat ta' l-estrazzjoni . Jekk ikun armat b' *siphon* (*Soxhlet apparatus*),ir- *reflux rate* ghandha tkun tali li tipproduci madwar 10 cikli kull minuta; jekk mhux tat-tip tas-*siphon*, ir-*reflux rate* ghandha tkun madwar 10 ml kull minuta.

4.2. *Extraction thimbles*, minghajr sustanzi li jinhallu fil-*light petroleum* u li ghandhom porozita' li hija konsistenti mar-rekwiziti f'punt 4.1.

4.3. Forn ghat-tnixxif, jew forn bil-vakum issetjat fuq 75 ºC ± 3 ºC jewforn ta' l-arja issetjat fuq 100 ºC ± 3 ºC.

5. **Procedura**

5.1 Procedura A (ara punt 8.1)

Izen 5g mil-kampjun sa l-eqreb 1mg, poggih f'*extraction thimble* (4.2) u aghttih b'bicca tajjara li ma tkunx xahmija.

Poggi t-*thimble* f' *extractor* (4.1) u estratta ghal 6 sighat bil-*light petroleum* (3.1). Igbor l-estratt tal-*light petroleum* fi flask *niexef u mwiezen* li fih frammenti tal-gebla haffiefa.[1]

Nehhi s-solvent bid-distillazzjoni . Nixxef ir-residwu billi zzomm il-*flask* fil-forn ghat-tnixxif ghal siegha u nofs (4.3). Hallih jiksah f'dessikatur u iznu. Erga nixfu ghal 30 minuta biex tkun cert li l-piz taz-zejt u x-xaham jibqa' kostanti (telf fil-piz bejn id-darbtejn li tizen m'ghandux ikun ta' iktar minn 1mg).

[1] Fejn iz-zejt jew ix-xaham irid jghaddi minn certu testijiet ta' kwalita', uza bococ tal-hgieg minflok frammenti tal-gebla haffiefa.

5.4 Procedura B

Izen 2.5g mil-kampjun sa l-eqreb 1mg (ara punt 8.2), poggih f'ibeaker tal-400ml jew *conical flask tat-300ml* u zid 100ml *hydrochloric acid* (3.3) u frammenti tal-gegla haffiefa. Aghtti l-*beaker* b'*watch glass* jew qabbad il-*conical flask* ma' *reflux condenser*. Halli t-tahlita tghalli bil-mod fuq fjamma baxxa jew *hot-plate* u zommha hemm ghal siegha. Thallix il-prodott jehel mal-gnub tal-kontenitur.

Kessah u zid kwantita' ta' *filtration aid* (3.4) bizzejjed biex jigi evitat telf ta' zejt u xaham waqt il-filtrazzjoni. Iffiltraha minn go *filter paper* doppja li tkun imxarrba u li ma tkunx xahmija. Ahsel ir-residwu b'ilma kiesah sakemm tottjeni filtrat newtrali. Ara li l-filtrat ma jkunx fih zejt jew xaham. Il-prezenza ta' dawn hija indikazzjoni li l-kampjun irid jigi estratt bil-*light petroleum*, permezz ta' Procedura A, qabel l-idrolisi.

Poggi d-*filter paper* doppja bir-residwu fuq *watch glass* u nixxifha ghal siegha u nofs fil-forn ta' l-arja (4.3) 100 ºC ± 3 ºC.

Poggi l-*filter paper* doppja bir-residwu niexef) u aghttih b'bicca tajjara li ma tkunx xahmija . Poggi t-*thimble* f' *extractor* (4.1) u kompli kif indikat fit-tieni u t-tielet paragrafi ta' punt 5.1.

6. **Espressjoni tar-rizultat**

Esprimi l-piz tar-residwu bhala persentagg tal-kamjun.

7. **Repetizzjoni**

Id-differenza bejn ir-rizultati ta' zewg determinazzjonijiet paralleli fuq l-istess kampjun mill-istess analista ma jistghux jeccedu :

0.2%, valur assolut, ghal kontenut ta' zjut mhux mahdumin u xahmijiet ta' inqas minn 5%;

4% irrelatati mal-oghla rizultat ghal kontenut ta' 5 sa 10%,

0.4%, valur assolut, ghal kontenut ta' iktar minn 10%

8. **Osservazzjonijiet**

8.1 Fil-kaz ta' prodotti b'kontenut gholi ta' zejt u xaham, li ma jistghux jitfarku facilment jew mhux adattati biex tottjeni kampjun ta' test ridott omogeniku, agmel dan li gej.

Izen 20g mil-kampjun sa l-eqreb 1mg u hallat ma' 10g jew iktar soluzzjoni ta' *anhydrous sodium sulfate (3.2).* Estratta bil-*light petroleum* (3.1) kif indikat f' punt 5.1. Imla l-estratt sal-marka ta' 500ml bil-ilight petroleum (3.1) u hallat. Hu 50ml mis-soluzzjoni u poggih f'*flask* zghir, niexef u mwiezen li fih frammenti ta' gebla

haffiefa.[1] Iddistilla s-solvent, nixxef u ipprocedi kif indikat fl-ahhar paragrafu taht punt 5.1.

Elimina s-solvent mir-residwu tal-estrazzjoni li jibqa' fit-*thimble*, farrak ir-residwu sakemm ikollu finezza ta' 1mm, poggih lura fl-*extraction thimble* (izzidx *sodium sulfate*) u ipprocedi kif indikat fit-tieni u t-tielet paragrafu ta' punt 5.1.

Ikkalkula l-kontenut ta' zejt u xaham bhala persentagg tal-kampjun permezz tal-formula:

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

Fejn :

a= massa fi grammi tar-residwu wara l-ewwel estrazzjoni (l-parti alikwota mill-estratt)

b= massa fi grammi tar-residwu wara t-tieni estrazzjoni

8.2 Fil-kaz ta' prodotti li fihom kontenut baxx ta' zejt u xaham il-kampjun tat-test jista' jigi mizjud ghal 5g.

8.3 Fil-kaz ta' ikel ghall-annimali li ghandhom kontenut gholi ta' ilma jista' jkun hemm bzonn li jithaltu ma' *anhydrous sodium sulfate* qabel l-idrolisi u l-estrazzjoni bhal Procedura B.

8.4 F'paragrafu 5.2 jista' jkun iktar effettiv jekk jintuza ilma jahraq minflok ilma kiesah biex jinhasel ir-residwu wara l-filtrazzjoni.

8.5 Jista' jkun li l-perjodu ta' tnixxif ta' siegha u nofs ikollu jigi estiz fil-kaz ta' cert ghalf. Ghandu jigi evitat tnixxif eccessiv ghax dan jista' jaghti rizultati baxxi. Jista' jintuza forn *microwave.*

8.6 L-estrazzjoni minn qabel b'procedura A qabel l-idrolisi u l-estrazzjoni mill-gdid b'procecura B ghandha tintuza jekk il-kontenut ta' zejt mhux mahdum jew xaham ikun oghla minn 15%. Sa certu punt dan jiddependi min-natura ta' l-ghalf u n-natura taz-zejt jew xaham fl-ghalf.

[1] [1] Fejn iz-zejt jew ix-xaham irid jghaddi minn certu testijiet ta' kwalita', uza bococ tal-hgieg minflok frammenti tal-*pumice stone.*

L.N. 99 of 2005

VETERINARY SERVICES ACT, 2001
(ACT XXIII OF 2001)

Methods of Analysis for the Official Control of Feedingstuffs Rules, 2005

IN exercise of the powers conferred by article 22 of the Veterinary Services Act, 2001 the Minister for Rural Affairs and the Environment has made the following regulations:-

Title, Scope and Applicability.

1. The title of these regulations is Methods of Analysis for Official Control of Feedingstuffs Regulations, 2004.

(2) The scope of these regulations is to implement the rules found under EU Commission Directive 71/393/EEC establishing EC methods for the official control of feedingstuffs.

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

SCHEDULE

I. DETERMINATION OF MOISTURE

1. Purpose and Scope

This method makes it possible 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 olaginous fruit defined in Council Regulation No 136/66/EEC (1) of 22 September 1966 on the establishment of a common organization of the market in oils and fats.

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

17 pages: Schedule

2. Principle

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

3. Apparatus

3.1. Crusher of non moisture-absorbing material which is easy to clean, allows rapid, even crushing without producing any appreciable heating, prevents contact with the outside air as far as possible and meets the requirements laid down in 4.1.1. and 4.1.2. (e.g. hammer or watercooled micro-crushers, collapsible cone mills, slow motion or cogwheeled 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 ºC) properly ventilated and ensuring rapid temperature regulation [3].

[3] (3) For the drying of cereals, flour, groats and meal, the oven must have a thermal capacity such that, when preset at 131 ºC, it will return to that temperature in less than 45 minutes after the maximum number of test samples have been placed inside to dry simultaneously. Ventilation must be such that, when as many samples of common wheat as it can contain are dried for two hours, the results differ from those obtained after four hours of drying by less than 0·15%.

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

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

4. **Procedure**

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

4.1. *Preparation*

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

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

4.1.2. *Cereals and groats*

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

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

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

4.2. *Drying*

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

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

leave to cool for 30 to 45 minutes in the desiccator (3.6) and weigh to the nearest 1 mg.

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

4.2.2. *Cereals, flour, groats and meal*

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

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

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

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

4.3. *Preliminary drying*

4.3.1. *Feedingstuffs other than those coming under 4.3.2*

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

Weigh 50 g of *uncrushed* sample to the nearest 10 mg (compressed or agglomerated feedingstuffs may be roughly divided if necessary) in a suitable container (e.g. a 20 × 12 cm aluminium plate with a 0·5 cm rim). Leave to dry in an oven from 60 ºC to 70 ºC until the moisture content has been reduced to between 8% and 12%. Remove from the oven, leave to cool uncovered in the laboratory for one hour and weigh to the 10 mg. Crush immediately as indicated in 4.1.1 and dry as indicated in 4.2.1 or 4.2.3 according to the nature of the feedingstuff.

4.3.2. *Cereals*

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

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

5. **Calculation of results**

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

5.1. *Drying without preliminary drying*

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

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

5.2. *Drying with preliminary drying*

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

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

M = mass, in grammes, of the test sample after preliminary drying,
M' = mass, in grammes, of the test sample after crushing or grinding,
m = mass, in grammes, of the dry test sample.

5.3. *Repeatibility*

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

6. **Observation**

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

II. **DETERMINATION OF VOLATILE NITROGENOUS BASES**

A. MICRODIFFUSION

1. **Purpose and Scope**

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

2. **Principle**

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

3. **Reagents**

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

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

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

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

3.5. Sulphuric acid 0·02 N.

4. **Apparatus**

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

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

4.3. Microburettes graduated in 1/100 ml.

5. **Procedure**

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

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

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

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

6. **Calculation of results**

1 ml of H2SO4 0·02 N corresponds to 0·34 mg of ammonia. Express the result as a precentage of the sample.

Repeatibility

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

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

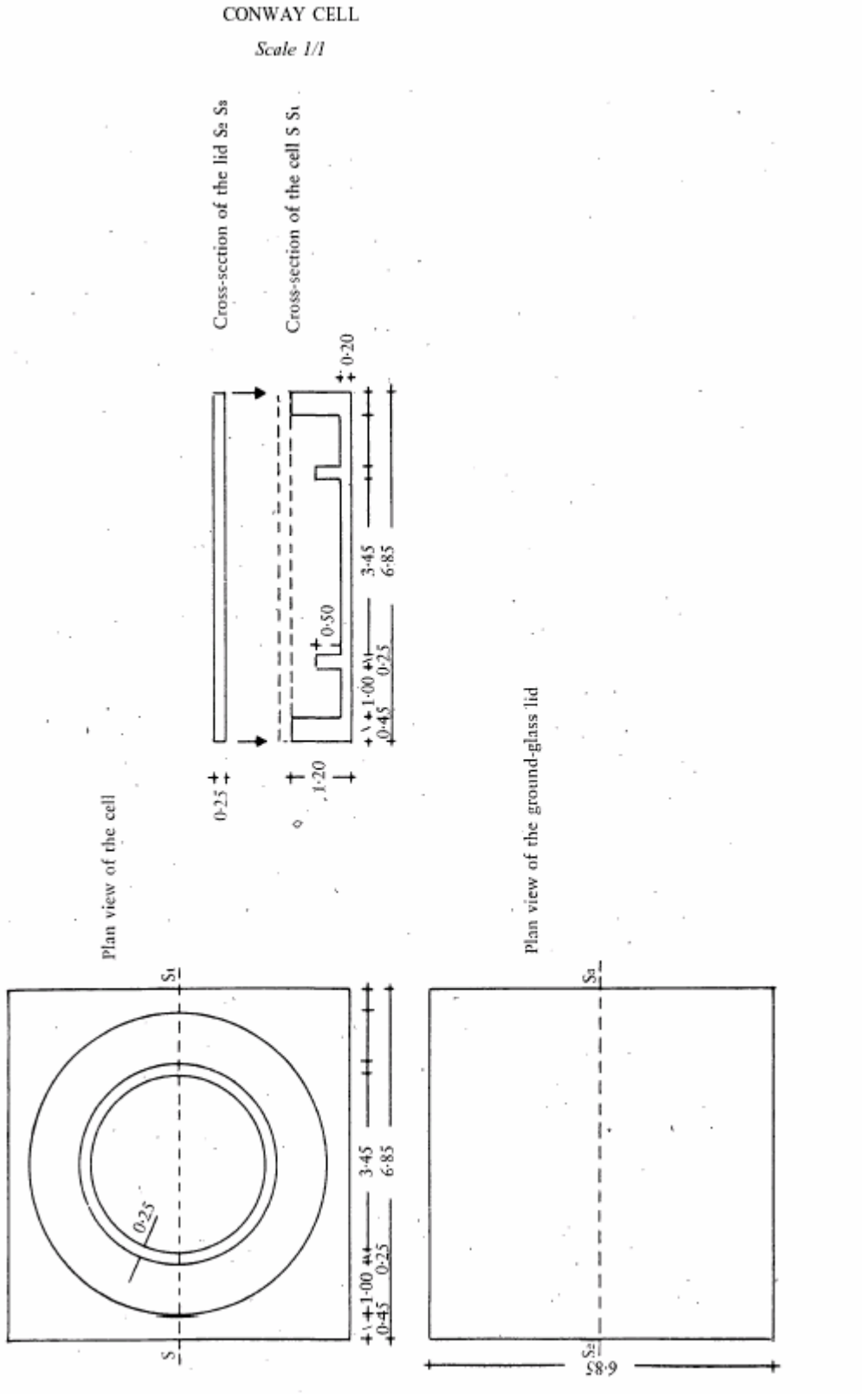
0·1%, in absolute value, for ammonia contents of 1·0% or more.

7. **Observation**

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

CONWAY CELL

Scale 1/1



B. BY DISTILLATION

1. Purpose and Scope

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

2. Principle

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

3. Reagents

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

3.2. Magnesium oxide A.R.

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

3.4. Sulphuric acid 0·1 N.

3.5. Sodium hydroxide solution 0·1 N.

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

4. Apparatus

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

4.2. Distilling apparatus of the Kjeldahl type.

5. Procedure

Weigh 10 g of the sample to the nearest 1 mg and place with 100 ml of water in a 200 ml graduated flask. Mix in the tumbler for 30 minutes. Add 50 ml of trichloroacetic acid solution (3.1), make upto 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 H2SO4 0·1 N corresponds to 1·7 mg of ammonia. Express the result as a percentage of the sample.

Repeatibility

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

III. DETERMINATION OF TOTAL PHOSPHORUS

PHOTOMETRIC METHOD

1. **Purpose and Scope**

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

2. **Principle**

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

3. **Reagents**

3.1. Calcium carbonate A.R.

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

3.3. Nitric acid A.R., d: 1·045.

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

3.5. Sulphuric acid A.R., d: 1·84.

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

3.6.1. Ammonium heptamolybdate solution: dissolve in hot water 100 g of ammonium heptamolybdate A.R. (NH4) 6Mo7O24.4H2O. Add 10 ml of ammonia (d: 0·91) and make up to 1 litre with water.

3.6.2. Ammonium monovanadate solution: 2·35 g of ammonium monovanadate A.R. NH4VO3 dissolve in 400 ml of hot water. Stirring constantly, slowly add 20 ml of dilute nitric acid (7 ml of HNO3 (3.4) + 13 ml of H2O and make upto 1 litre with water.

3.7. Standard solution of 1 mg phosphorus per ml: dissolve 4·387 g of potassium dihydrogen phosphate A.R. KG2PO4 in water. Make up to 1 litre with water.

4. **Apparatus**

4.1. Silica or porcelain ashing crucibles.

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

4.3. 250 ml Kjeldahl flask.

4.4. Graduated flasks and precision pipettes.

4.5. Spectrophotometer.

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

5. **Procedure**

5.1. *Preparation of the solution*

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

5.1.1. *Usual procedure*

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

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

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

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

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

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

5.3. *Calibration curve*

From the standard solution (3.7) prepare solutions containing respectively 5, 10, 20, 30 and 40 µg of phosphorus per ml. Take 10 ml of each of these

solutions and add thereto 10 ml of molybdovanadate reagent (3.6). Homogenise and leave to stand for at least 10 minutes at 20 ºC. Measure the optical density as indicated in 5.2. Trace the calibration curve by plotting the optical densities against the corresponding quantities of phosphorus. For concentrations between 0 and 40 µg/ml, the curve will be linear.

6. **Calculation of results**

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

Express the result as a percentage of the sample.

Repeatibility

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

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

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

IV. DETERMINATION OF CRUDE OILS AND FATS

1. Purpose and scope

This method is for the determination of crude oils and fats in feedingstuffs. It does not cover the analysis of oil seeds and oleaginous fruit defined in Council Regulation 136/66/EEC of 22 September 1966.

The use of the two procedures described below depends on the nature and composition of the feedingstuff and the reason for carrying out the analysis.

1.1. *Procedure A — Directly extractable crude oils and fats*

This method is applicable to feed materials of plant origin, except those included within the scope of Procedure B.

1.2. *Procedure B — Total crude oils and fats*

This method is applicable to feed materials of animal origin and to all compound feeds. It is to be used for all materials from which the oils and fats cannot be completely extracted without prior hydrolysis (eg glutens, yeast, potato proteins and products subject to processes such as extrusion, flaking and heating).

1.3. *Interpretation of results*

In all cases where a higher result is obtained by using Procedure B than by Procedure A, the result obtained by Procedure B shall be accepted as the true value.

2. **Principle**

2.1. *Procedure A*

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

2.2. *Procedure 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 Procedure A.

3. **Reagents**

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

3.2. Sodium sulfate, anhydrous.

3.3. Hydrochloric acid, c = 3 mol HCl/l

3.4. Filtration aid, e.g. Kieselguhr, 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 ºC ± 3 ºC or an air-oven set at 100 ºC ± 3 ºC.

5. **Procedure**

5.1. *Procedure A (see point 8.1)*

Weigh 5 g of the sample to the nearest 1 mg, 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 stone [1].

Distil off the solvent. Dry the residue maintaining the flask for one and a half hours in the drying oven (4.3). Leave to cool in a desiccator 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. *Procedure B*

Weigh 2,5 g of the sample to the nearest 1 mg (see point 8.2), place in a 400 ml beaker or a 300 ml conical flask and add 100 ml of hydrochloric acid (3.3) and fragments of pumice stone. Cover the beaker with a watch glass or fit the conical flask with a reflux condenser. Bring the mixture to a gentle boil over a low flame or a hot-plate and keep it there for one hour. Do not allow the product to stick to the sides of the container.

Cool and add a quantity of filtration aid (3.4) sufficient to prevent any loss of oil and fat during filtration. Filter through a moistened, fat-free, double filter paper. Wash the residue in cold water until 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 Procedure 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 air oven (4.3) 100 ºC ± 3 ºC.

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:

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

— 0,2 % in absolute value, for contents of crude oils and fats lower than 5 %,

— 4,0 % relative 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 sulfate (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 mix. Take 50 ml of the solution and place in a small, dry, weighed flask containing fragments of pumice stone [1].

Distil off the solvent, dry and proceed as indicated in the last paragraph of 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 sulfate) 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:

$(10\,a + b) \times 5$

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.

8.3. Pet foods containing a high content of water may need to be mixed with anhydrous sodium sulfate prior to hydrolysis and extraction as per Procedure B.

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

8.4. In paragraph 5.2 it may be more effective to use hot water in place of cold water to wash the residue after filtration.

8.5. The drying time of 1,5 h may need to be extended for some feedingstuffs. Excessive drying should be avoided as this can lead to low results. A microwave oven can also be used.

8.6. Pre-extraction by Procedure A prior to hydrolysis and re-extraction by Procedure B is recommended if the crude oil/fat content is greater than 15 %. To some extent this depends on the nature of the feedingstuff and the nature of the oil-fat in the feedingstuff.

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