オ．　発酵脱皮大豆油かすの暫定値申請資料

１　暫定申請値

単位：％（ＭＥはＭｃａｌ／ｋｇ）

申請飼料名	組成（原物中）						消化率及び栄養価											備考
							鶏		豚					牛				
	水分	粗たん白質	粗脂肪	ＮＦＥ	粗繊維	粗灰分	代謝率	ＭＥ	粗たん白質	粗脂肪	ＮＦＥ	粗繊維	ＴＤＮ	粗たん白質	粗脂肪	ＮＦＥ	粗繊維	ＴＤＮ	
発酵脱皮大豆油かす	7.0	57.9	0.4	24.3	3.2	7.1			92	40	98	87	80.2						脱皮大豆油かすを枯草菌で発酵処理したものであること。栄養価は、暫定的に定めるものである。
試料数	20	20	20	20	20	20													
標準偏差	0.8	0.8	0.0	0.9	0.3	0.2													
最大値	8.4	59.0	0.5	26.4	3.8	7.6													
最小値	5.5	56.6	0.4	23.0	2.8	6.8													

２　類似する飼料の既存の日本標準飼料成分表及び暫定値

単位：％（ＭＥはＭｃａｌ／ｋｇ）

原料名	組成（原物中）						消化率及び栄養価											備考
							鶏		豚					牛				
	水分	粗たん白質	粗脂肪	ＮＦＥ	粗繊維	粗灰分	代謝率	ＭＥ	粗たん白質	粗脂肪	ＮＦＥ	粗繊維	ＴＤＮ	粗たん白質	粗脂肪	ＮＦＥ	粗繊維	ＴＤＮ	
発酵脱皮大豆油かす	11.6	53.3	1.6	22.1	4.2	7.2			89	98	86	91	73.8						脱皮大豆油かすをアスペルギルス アワモリで発酵処理したものであること。
発酵脱皮大豆油かす	6.0	52.6	1.1	30.7	3.1	6.5			95	40	86	39	78.6						脱皮大豆油かすを乳酸菌で発酵処理したものであること。

原材料のTDN又はMEに関する申請書

１．名称	発酵脱皮大豆油かす
２．定義	脱皮大豆油かすに枯草菌を添加し、発酵過程を通して生産された製品
３．製造方法及び製造工程	脱皮大豆油かすを枯草菌により発酵処理し、乾燥、粉砕したもの
４．対象家畜 （1）使用目的 （2）使用割合又は使用量	（１）豚用 （２）1～30%添加

５．成分量
（1）一般成分

水分	粗たん白質	粗脂肪	可溶無窒素物	粗繊維	粗灰分	総エネルギー	備　考
7.0%	57.9%	0.4%	24.3%	3.2%	7.1%	4.54Mcal/kg	

（2）消化率
可消化成分

鶏							豚					牛					備 考
CP	Fat	Fib	NFE	TDN	代謝率	ME	CP	Fat	Fib	NFE	TDN	CP	Fat	Fib	NFE	TDN	
							92%	40%	87%	98%	80.2%						

（3）特殊成分	
６．備考	発酵菌として枯草菌を利用した。

発酵脱皮大豆油かす

分析法：ＡＯＣＳ法
分析機関名：CJ Cheil Jedang Corp　仁川第二工場

単位：%

試料番号	水分	ＣＰ	ＥＥ	ＮＥＦ	粗繊維	粗灰分
1	6.5	56.9	0.4	26.4	3.0	6.9
2	6.3	57.5	0.4	25.3	3.0	7.6
3	7.9	57.3	0.4	23.6	3.6	7.2
4	6.9	58.1	0.5	24.0	3.4	7.2
5	7.2	57.0	0.4	25.5	3.0	7.0
6	7.3	56.9	0.4	25.3	2.8	7.3
7	6.8	58.4	0.5	24.4	2.8	7.1
8	7.2	58.5	0.4	23.4	2.9	7.1
9	7.0	58.2	0.4	24.2	3.2	7.2
10	5.5	57.9	0.4	25.9	3.3	7.2
11	5.6	58.8	0.4	24.7	3.2	7.3
12	7.2	58.9	0.4	23.0	3.3	7.2
13	8.4	57.3	0.5	23.9	3.0	7.0
14	7.5	58.2	0.4	23.8	3.0	7.1
15	7.8	57.4	0.4	24.0	3.2	7.2
16	7.9	57.3	0.4	24.1	3.1	7.2
17	8.1	56.6	0.4	24.5	3.5	6.9
18	6.6	58.8	0.4	24.4	3.4	6.8
19	6.7	59.0	0.4	23.5	3.5	7.1
20	6.7	58.8	0.4	23.0	3.8	7.2
平　均	7.0	57.9	0.4	24.3	3.2	7.1
標準偏差	0.8	0.8	0.0	0.9	0.3	0.2
最大値	8.4	59.0	0.5	26.4	3.8	7.6
最小値	5.5	56.6	0.4	23.0	2.8	6.8

科飼協試報 22-181 号
試験コード番号：S-10-S003
平成 22 年 7 月 26 日

発酵大豆粕の豚における消化率および栄養価の測定

社団法人 [redacted] 料協会
青木 健、[redacted] 貴三千人
米持千里

要 約

「飼料及び飼料添加物の成分規格等に関する省令の一部を改正する省令等の施行について（昭和 56 年 7 月 27 日付け、56 畜 B 第 1594 号）」による「飼料の品質表示に係る可消化粗たん白質、可消化養分総量又は代謝エネルギーの取扱い」に定める消化試験法に準じて、発酵大豆粕の豚における消化率、可消化養分総量及び可消化エネルギーを測定した。その結果は、以下に示したとおりであった。

発酵大豆粕の一般成分及び総エネルギー

水分 (%)	粗たん白質 (%)	粗脂肪 (%)	可溶無窒素物 (%)	粗繊維 (%)	粗灰分 (%)	総エネルギー (Mcal/kg)
7.6	58.2	0.6	23.4	3.0	7.2	4.54

発酵大豆粕の消化率（%）

粗たん白質	粗脂肪	可溶無窒素物	粗繊維	エネルギー
92.4±2.4	40.0±36.5	98.0±2.5	86.7±7.5	93.2±2.0

注）平均値±標準偏差（n=5）

発酵大豆粕の消化率及び栄養価

可消化養分総量（%）	可消化エネルギー（Mcal/kg）
79.9±1.2	4.23±0.10

注）平均値±標準偏差（n=5）

1、目　的

「飼料及び飼料添加物の成分規格等に関する省令の一部を改正する省令等の施行について（昭和56年7月27日付け、56畜B第1594号）」による「飼料の品質表示に係る可消化粗たん白質、可消化養分総量又は代謝エネルギーの取扱い」に定める消化試験法に準じて、発酵大豆粕の豚における消化率、可消化養分総量（TDN）及び可消化エネルギー（DE）を測定する。

2、材料及び方法

1）供試品

あすか製薬株式会社より提供された発酵大豆粕を供試した。

2）供試動物

体重36.5～42.6kg（平均39.3kg）のLW･D種去勢子豚を10頭供試した。

3）試験区の設定等

表1に示した基本飼料を給与する基本飼料給与区と、基本飼料と供試品を8：2の割合で混合した試験飼料を給与する試験飼料給与区の計2区を設定した。なお、基本飼料及び試験飼料とも、指示物質として酸化クロム（Cr_2O_3）を0.1%ずつ混合した。

表1　基本飼料の配合割合（%）

原　料	配合割合
トウモロコシ	56.70
マイロ	20.00
脱脂米ぬか	15.00
魚粉	5.00
炭酸カルシウム	1.50
リン酸ニ石灰	0.80
食塩	0.40
ビタミンB群[1)]	0.20
ビタミンADE[2)]	0.20
微量ミネラル[3)]	0.20
計	100.00

注1）1kg中；硝酸チアミン1.0g、リボフラビン7.0g、塩酸ピリドキシン0.5g、ニコチン酸アミド6.0g、D-パントテン酸カルシウム10.9g、塩化コリン57.60g
2）1g中；ビタミンA 10,000IU、ビタミンD_3 2,000IU、酢酸dl-α-トコフェロール10mg
3）1kg中；Mn 50g、Fe 50g、Cu 10g、Zn 60g、I 1g

供試豚を個体別に代謝試験用ケージに収容し、全頭に基本飼料を7日間給与して試験

環境に馴致させたのち、基本飼料あるいは試験飼料を 5 頭ずつに割り付け、各供試飼料を 10 日間定量給与した。飼料給与量は各供試豚の区分け時体重の約 3.3%量とし、朝、夕の 2 回に分けて等量ずつ給与した。

両飼料給与開始後 6 日目より 5 日間に排泄された新鮮糞を毎日、朝、夕の 2 回、個体毎に採取した。採取した糞は、その都度、秤量後、全量を約 60℃で 2 日間通風乾燥し、風乾したのち、5 日分を混合して微粉砕し、分析用試料とした。

4）分析

供試品は、飼料の安全性の確保及び品質の改善に関する法律施行規則（昭和 51 年 7 月 24 日、農林省令第 36 号）の規定に基づく分析方法により、一般成分〔水分、粗たん白質（CP）、粗脂肪、粗繊維、粗灰分及び可溶無窒素物（NFE）〕を分析するとともに、ボンブカロリーメーターを用いて総エネルギー（GE）を分析した。

基本飼料、試験飼料及び採取した糞は、前述の方法により一般成分及び GE を分析するとともに、比色法[1]により Cr_2O_3 を分析した。

5）消化率及び栄養価の計算

Cr_2O_3 を指示物質としたインデックス法の計算式[2]を用いて、基本飼料及び試験飼料の各成分消化率を計算したのち、以下の式を用いて、供試品の消化率、TDN 及び DE を算出した。

$$\text{供試品の消化率 (\%)} = \frac{\text{試験飼料の可消化成分含量} - \text{基本飼料の可消化成分含量} \times \text{基本飼料の混合割合（80\%）}}{\text{供試品の成分含量} \times \text{供試品の混合割合（20\%）}}$$

$$\text{供試品のTDN (\%)} = \text{（供試品の CP×供試品の CP 消化率+供試品の粗脂肪×供試品の粗脂肪消化率×2.25+供試品の粗繊維×供試品の粗繊維消化率+供試品の NFE×供試品の NFE 消化率）/100}$$

$$\text{供試品のDE (Mcal/kg)} = \text{供試品の GE×供試品の GE 消化率}$$

6）試験実施期間（飼育期間）

平成 22 年 6 月 15 日～7 月 2 日

4、試験結果

供試品の一般成分及び GE は表 2 に、消化率及び栄養価は表 3 に示したとおりであっ

た。

表２　供試品の一般成分及び総エネルギー

水分（%）	CP（%）	粗脂肪（%）	NFE（%）	粗繊維（%）	粗灰分（%）	GE (Mcal/kg)
7.6	58.2	0.6	23.4	3.0	7.2	4.54

表３　供試品の消化率及び栄養価（%）

消化率（%）					栄養価	
CP	EE	NFE	粗繊維	エネルギー	TDN（%）	DE (Mcal/kg)
92.4±2.4	40.0±36.5	98.0±2.5	86.7±7.5	93.2±2.0	79.9±1.2	4.23±0.10

注）平均値±標準偏差（n=5）

5、参考文献

1）武政正明；リン酸カリ試薬による酸化クロム定量法の改良、畜産試験場研究報告 52（1992）

2）独立行政法人 農業・食品産業技術総合研究機構編；日本標準飼料成分表（2009 年版）、（社）中央畜産会（2010）

付表１　供試品、供試飼料及び糞の分析値

試料		水分 (%)	CP (%)	EE (%)	NFE (%)	粗繊維 (%)	粗灰分 (%)	GE (Mcal/kg)	Cr_2O_3 (%)
供試品		7.6	58.2	0.6	23.4	3.0	7.2	4.54	－
基本飼料	飼料	11.7	11.5	3.1	65.1	2.4	6.2	3.77	0.098
	1	11.5	16.5	6.3	32.8	10.0	22.9	3.67	0.572
	2	11.3	14.9	4.9	35.2	9.9	23.8	3.67	0.583
	3	10.9	16.4	5.7	33.4	9.9	23.7	3.73	0.566
	4	11.7	14.6	4.3	36.3	10.0	23.1	3.75	0.576
	5	11.4	14.7	5.4	35.7	10.1	22.7	3.80	0.585
試験飼料	飼料	10.9	20.8	2.6	56.8	2.5	6.4	3.92	0.098
	6	11.6	18.9	5.0	30.0	9.6	24.9	3.63	0.639
	7	12.1	20.8	5.9	28.8	9.1	23.3	3.69	0.613
	8	11.3	16.7	5.3	32.4	9.3	25.0	3.63	0.644
	9	11.6	19.2	4.8	31.7	9.2	23.5	3.69	0.635
	10	11.6	19.5	5.2	31.4	9.1	23.2	3.68	0.653

注）試験飼料の成分値は供試品及び基本飼料からの計算値

付表２　供試飼料の消化率（%）

区	個体番号	CP	EE	NFE	粗繊維	エネルギー
基本飼料	1	75.5	65.4	91.4	29.0	83.4
	2	78.3	73.6	91.0	31.0	83.7
	3	75.4	68.3	91.2	28.9	83.0
	4	78.5	76.5	90.6	29.5	83.2
	5	78.7	71.0	90.9	29.9	83.2
	平均	77.3	71.0	91.0	29.7	83.3
試験飼料	6	86.1	70.7	91.9	41.4	85.9
	7	84.1	63.9	91.9	42.1	85.0
	8	87.8	69.1	91.4	43.7	86.0
	9	85.8	71.7	91.4	43.5	85.5
	10	86.0	70.1	91.7	45.7	86.0
	平均	86.0	69.1	91.7	43.3	85.7

付表 3　供試品の消化率（%）

個体番号	CP	EE	NFE	粗繊維	エネルギー
6	92.6	33.3	100.0	73.3	94.5
7	89.2	0.0	100.0	90.0	90.1
8	96.0	33.3	95.3	90.0	94.5
9	91.8	100.0	95.3	90.0	92.3
10	92.6	33.3	99.6	90.0	94.5
平均	92.4	40.0	98.0	86.7	93.2

付表 4　供試品の栄養価

個体番号	DE（Mcal/kg）	TDN（%）
6	4.29	79.9
7	4.09	78.0
8	4.29	81.3
9	4.19	79.8
10	4.29	80.3
平均	4.23	79.9

分析法＜訂正版＞

1. 水分（韓国・食品公定法）

常圧加熱乾燥法

105℃前後で 3～5 時間乾燥後、デシケーターで冷却して重量を量る。再び、1～2 時間乾燥させて、恒量になるまで繰り返す。この乾燥蒸発分を百分率で表す。

2. 粗たん白（AOCS Ba-4d-90）※別紙参照

① 分析する試料を粉砕する（Cyclotec sample mill で 1mm 通過）
② 試料をパラフィンで定秤し kjeltec tube へ入れる
③ 触媒剤（K2SO4+Se）を投入する（触媒 1000:1）
④ H2SO4 を入れ、H2O2 で分解を促進させる
⑤ Tube を分解定置である加熱された Heater に入れ、分解させる（所要時間約 1 時間、明るい色→黒色→黄色→明るい色）
⑥ 分解が完了した tube を Auto Kjeltec System へ挿入し、分析を行う

3. 粗脂肪（AOAC 991.36）※別紙参照

① Thimble を使用して試料を Chemical Balance で定秤する
② Soxtec 分析定置を使用して分析を行う
③ 抽出完了後、抽出された脂肪酸を測定して飼料中の油分量を百分率で求める

※ Soxtec 分析とは

Soxhlet 原理を基礎にして機械化、自動化したことにより抽出時間を短縮できる。
一般的に 8～16 時間かかる抽出時間を 30 分～1 時間に短縮可能。（抽出方法は Boiling---Rinsing---Recovery の 3 段階からなる）

4. 粗繊維（AOCS Ba-6-84）※別紙参照

① Chemical Balance で試料を定秤する
② 加温された 1.25%H2SO4 を入れ、30 分間火にかける
③ 加温された 1.25%NaOH を入れ、30 分間火にかける
④ 酸、アルカリ処理された重量W0 を測定する
⑤ 600 度の電気炉で 2 時間灰化後、Dessicator で 30 分間冷却後、秤量する

5. 粗灰分（AOCS Ba-5a-49）※別紙参照

電気灰化炉 600 度で灰化後、灰化された有機物に重さを百分率で表す

AOCS Official Method Ba 4d-90

Revised 2003

Nitrogen–Ammonia–Protein Modified Kjeldahl Method Titanium Dioxide + Copper Sulfate Catalyst

DEFINITION

This method determines total nitrogen content and protein as 6.25 times the nitrogen content of the sample (see Notes, 1 and References, 1).

SCOPE

Applicable to cottonseed meats, and meal and ground cake or pellets from cottonseed, soybeans, peanuts and flaxseed.

APPARATUS

1. Kjeldahl digestion and distillation apparatus—complete with heat source, traps and block-tin, or equivalent noncorrosive tubing condensers (see Notes, 2).
2. Kjeldahl flasks—800 mL.
3. Distillate receiving flasks—500 mL, or any convenient size.

REAGENTS ✓

1. Catalyst mixture—containing potassium sulfate, 16.7 g + titanium dioxide, 0.6 g + copper sulfate, 0.01 g + pumice, 0.3 g.
2. Sulfuric acid—sp. gr. 1.84 (see Notes, *Caution*).
3. Sodium hydroxide solution—sp gr 1.50 (see Notes, *Caution*).
4. Sodium hydroxide solution—0.25 N, accurately standardized (see AOCS Specification H 12-52).
5. Sulfuric acid—0.5 N, accurately standardized (see AOCS Specification H 13-52).
6. Methyl red indicator solution—0.1% in ethyl alcohol, or Alizarin Red S—0.3% in distilled water.
7. Alundum boiling stones—8–14 mesh.
8. Zinc metal—granular. (If the catalyst is used with added pumice, granular zinc metal need not be added as an antibumping agent.)

PREPARATION OF SAMPLE

1. Grind the 100-g portion of sample from AOCS Official Method Ba 2a-38, Preparation of Sample, through a suitable laboratory mill to a uniform fineness of about 20 mesh (about 30 mesh for linseed meal). Mix the sample thoroughly. A Henry velocity mixer (available from Mid-Continent Engineering, Memphis, TN, USA) may be used, or mixing may be done by placing the entire ground sample into a 0.5-gallon Mason fruit jar containing a large rubber stopper. Replace the cover and shake vigorously until thoroughly mixed. Transfer the ground sample to an airtight container. Oil, second (ground) moisture and protein nitrogen are determined on this ground portion. Weighings for all determinations should be made at the same time. If determining ground moisture, immediately proceed with the moisture determination.

PROCEDURE

1. Weigh 0.250–1.750 g of the ground sample (see Notes, 3) into the Kjeldahl flask. Add the catalyst mixture (premixed catalyst packs are commercially available and may be used).
2. Add 30 mL of concentrated H_2SO_4 (see Notes, 4) to the sample and catalyst in the digestion flask. Include at least one sample of high-purity lysine hydrochloride in each day's run as a check of the correctness of digestion parameters. If recovery is not complete, make appropriate adjustments.
3. Add a few Alundum boiling stones to the sample flask.
4. To digest the sample, first adjust heat to bring 250 mL of water at 25°C to rolling boil in 5 min. Place the sample flask on the digestion rack in an inclined position and then heat sample flask at the 5-min boil rate until dense, white fumes clear bulb of flask (approximately 5 min). Swirl gently and continue heating an additional 40 min after the liquid has become clear and colorless (see Notes, 5). The Kjeldahl flasks should be rotated a minimum of three times during the digestion.
5. Cool, cautiously add about 300 mL of water and cool to room temperature (see Notes, 6). Add water as soon as possible to reduce amount of caking. If excessive bumping occurs during distillation, increase dilution water from 300 mL to about 350 mL.
6. Accurately transfer a sufficient quantity of the standard acid into receiving flask so that there will be an excess of at least 0.5 mL of 0.5 N acid. Add sufficient distilled water to cover the end of the outlet tube and attach to outlet end of condenser tube. The distillate should discharge through a glass tube at the bottom of the receiving flask.
7. Add an additional 0.5–1.0 g Alundum boiling stones to cooled digestion flask. Mix thoroughly and add sufficient alkali solution (Reagents, 3) to make strongly alkaline. Pour the alkali down the side of the Kjeldahl flask so that it does not mix quickly with the acid.
8. Immediately connect the Kjeldahl flask to the other end of the condenser tube and thoroughly mix the contents by shaking. Apply heat at about a 7.5-min boil rate and distill until at least 150 mL of distillate have been collected.
9. Titrate the contents of the receiving flask with 0.25 N NaOH solution, using 3 or 4 drops of indicator.

10. Conduct a blank determination of all reagents simultaneously with the samples and similar in all respects. Correct for blank determined on reagents.
11. The performance of the entire method should be checked frequently by analyzing either National Institute of Standards and Technology (NIST) certified ammonium dihydrogen phosphate [Standard Reference Material (SRM) 194] or high-purity lysine hydrochloride.
12. Determine moisture in the ground sample as directed in AOCS Official Method Ba 2a-38, Procedure section.

CALCULATIONS

1. Nitrogen, % =

$$\frac{[(NA \times mLA) - (mLBK \times NB) - (mLB \times NB)] \times 1400.67}{mg\ sample}$$

Where—

mLB = volume, mL standard base used for the sample
mLA = volume, mL standard acid used for that sample
mLBK = volume, mL standard base needed to titrate 1 mL of standard acid minus mL standard base needed to titrate reagent blank carried through method and distilled into 1 mL standard acid
NA = normality of standard acid
NB = normality of standard base

Note—To determine percent ammonia, substitute 1703.06 for 1400.67 in the equation for the calculation of percent nitrogen

2. Refer to the Conversion Table for Nitrogen, Ammonia and Protein in AOCS Official Method Aa 5-91 for conversion from nitrogen (based on conversion factor of 6.25) or ammonia (based on conversion factor of 5.14) to protein.

PRECISION

1. Table 1 lists the statistical parameters for soybean meal and soy protein concentrate determined from an Association of Official Analytical Chemists (AOAC) interlaboratory study.
2. Tables 2, 3 and 4 list the results of the second AOCS collaborative study, March 1991, for the determination of nitrogen in cottonseed and cottonseed meal, using a 0.56 catalyst to acid ratio for $TiO_2/CuSO_4$ catalyst. The project coordinator was James Falk, member of the AOCS Seed and Meal Analysis Technical Committee.

NOTES

Caution

Sulfuric acid is a strong acid and will cause severe burns. Protective clothing should be worn when working with this acid. It is an oxidizing agent and should not be stored in the vicinity of organic materials. Use great caution in mixing with water due to heat evolution that can cause explosive spattering. Always add the acid to water, never the reverse.

Alkalies can burn skin, eyes and respiratory tract severely. Wear heavy rubber gloves and face shield to protect against concentrated alkali solutions. Use effective fume-removal device or gas mask to protect respiratory tract against alkali dusts or vapors. When working with extremely caustic materials like sodium hydroxide and potassium hydroxide, always add pellets to water and not vice versa. These alkalies are extremely exothermic when mixed with water. Take precautions to contain the caustic solution in the event the mixing container breaks from the extreme heat generated.

NUMBERED NOTES

1. This method has been evaluated through interlaboratory comparison of catalysts and has been adopted by the committee as the official replacement for the mercuric oxide catalyzed method, AOCS Method Ba 4a-38. An interlaboratory evaluation (References, 3) indicated that AOCS Official Method Ba 4d-90 (which uses the copper/titanium catalyst mixture) produces results more closely in agreement with AOCS Official Method Ba 4a-38 (using mercuric oxide catalyst) than methods using copper sulfate catalyst. As a result of this study, Methods Ba 4a-38 (mercuric oxide), Ba 4b-87 (copper sulfate) and Ba 4c-87 (Kjel–Foss Automatic) were declared surplus (obsolete) in 1991.

The AOAC collaborative study (References, 4) showed no bias when comparing Cu/Ti catalyst with HgO. The AOCS interlaboratory evaluation (References, 3), based on sample weight, acid, volume, digestion temperature and time, previously specified in AOCS Official Method Ba 4a-38, indicated an average bias of –0.244%, which was statistically significant at the 99% confidence level. Factors that may affect method bias are sample weight and particle size, catalyst composition, use of Alundum, volume of acid used for digestion, digestion temperature and digestion time. In 1991, two additional collaborative studies (cottonseed

Table 1
Statistical parameters determined from the AOAC interlaboratory study.[a]

	Soybean meal				Soy protein concentrate			
	1		2		1		2	
	Ti-Cu	HgO	Ti-Cu	HgO	Ti-Cu	HgO	Ti-Cu	HgO
Labs	13	13	13	13	13	13	13	13
Labs removed	1	1	2	1	2	0	2	1
% protein	48.76	48.58	48.11	48.02	87.13	87.27	88.70	88.69
r_1CV%	0.88	1.06	0.60	0.41	0.44	0.75	0.43	0.74
R_1CV%	0.88	1.08	0.99	0.96	0.76	0.89	0.58	0.74

[a]References, 4

Table 2
Summary of collaborative study results for determination of percent nitrogen (dry-weight basis) in delinted cottonseed with 21.2% oil content, using 30 mL of acid for digestion.

Sample	1		9		1 + 9	
	Ti-Cu	HgO	Ti-Cu	HgO	Ti-Cu	HgO
% Nitrogen	4.28	4.29	4.31	4.30	4.30	4.30
SD	0.04	0.06	0.06	0.06	0.04	0.04
CV%	0.97	1.31	1.43	1.43	0.99	0.87
Range	0.13	0.22	0.18	0.20	0.13	0.12

Table 3
Summary of collaborative study results for determination of percent nitrogen (dry-weight basis) in high-protein cottonseed meal, using 30 mL of acid for digestion.

Sample	1		2		1 + 2	
	Ti-Cu	HgO	Ti-Cu	HgO	Ti-Cu	HgO
% Nitrogen	7.82	7.78	7.82	7.80	7.82	7.79
SD	0.09	0.09	0.07	0.05	0.07	0.06
CV%	1.14	1.15	0.90	0.64	0.90	0.73
Range	0.27	0.33	0.19	0.17	0.20	0.17

Table 4
Summary of collaborative study results for determination of percent nitrogen (dry-weight basis) in 52% protein cottonseed meal, using 30 mL of acid for digestion.

Sample	4		8		4 + 8	
	Ti-Cu	HgO	Ti-Cu	HgO	Ti-Cu	HgO
% Nitrogen	8.24	8.26	8.29	8.25	8.26	8.25
SD	0.23	0.09	0.06	0.09	0.14	0.08
CV%	0.97	0.34	0.20	0.30	0.59	0.30
Range	0.27	0.33	0.19	0.17	0.20	0.17

and cottonseed meal) showed that when 30 mL of sulfuric acid were used, caking of the digest was reduced and results were comparable to the mercuric oxide catalyzed method. As a result, the AOCS versions of this method may specify the use of more than 20 mL of sulfuric acid, but otherwise conform with the AOAC version (References, 5).

2. Use of steam- and water-resistant rubber tubing and stoppers to connect the glass parts is strongly recommended.
3. Due to large particle size, a larger sample, in the range of 1.4 to 1.7 g, is recommended for cottonseed meal. This may also require an adjustment in the volume of sulfuric acid used for sample digestion.
4. More than 20 mL of concentrated sulfuric acid is needed for samples high in fat or oil, because more acid is required to effect complete digestion, avoid loss in nitrogen and prevent caking. In recent collaborative studies involving the application of this method to cottonseed meal, it was observed that when 30 mL of H_2SO_4 was used, caking was reduced and results were comparable to the mercuric oxide catalyzed method.
5. Complete conversion of organic nitrogen to ammoniacal nitrogen is essential to obtain accurate and precise results. After the liquid has become clear and colorless, it may be necessary to digest the sample for approximately an additional 30–50 min. Reagent proportions, heat input and digestion time are critical factors and should not be changed. Prolonged digestion and high temperatures must be avoided, because some ammoniacal nitrogen can be lost by oxidation. Large sample particles should be avoided, because the larger particles will require a longer digestion time.
6. The cooled digest should be liquid, or liquid with a few small crystals. Caking before the addition of water indicates too little residual acid at the end of the digestion period and may result in low nitrogen values.

REFERENCES

1. Jones, D.B., USDA Circular No. 183, United States Department of Agriculture, 1941.
2. *Composition of Foods*, Agriculture Handbook No. 8, United States Department of Agriculture, Sections 11 and 12.
3. *INFORM 1*:884 (1990).
4. Kane, P.F., *J. Assoc. Off. Anal. Chem. 70*:907 (1987).
5. *Official Methods of Analysis*, AOAC International, 16th ed., Vol. 1, Gaithersburg, MD, 1995, Chapt. 4, p.13, Method 988.05.

39.1.08

AOAC Official Method 991.36
Fat (Crude) in Meat and Meat Products
Solvent Extraction (Submersion) Method
First Action 1991
Final Action 1996

[Applicable to meat and meat food products that can be analyzed using **960.39** (*see* 39.1.05), **976.21** (*see* 39.1.06), and **985.15** (*see* 39.1.07).]

Results of the interlaboratory study supporting acceptance of the method:

$\bar{x}$, 4.34% fat: $s_r = 0.106$; $s_R = 0.112$; $RSD_r = 2.44\%$; $RSD_R = 2.59\%$

$\bar{x}$, 27.29% fat: $s_r = 0.534$; $s_R = 0.637$; $RSD_r = 1.95\%$; $RSD_R = 2.33\%$

$\bar{x}$, 27.95% fat: $s_r = 0.648$; $s_R = 0.793$; $RSD_r = 2.32\%$; $RSD_R = 2.84\%$

$\bar{x}$, 34.51% fat: $s_r = 0.764$; $s_R = 0.799$; $RSD_r = 2.21\%$; $RSD_R = 2.31\%$

$\bar{x}$, 33.57% fat: $s_r = 0.340$; $s_R = 0.516$; $RSD_r = 1.01\%$; $RSD_R = 1.53\%$

$\bar{x}$, 26.20% fat: $s_r = 0.406$; $s_R = 0.613$; $RSD_r = 1.55\%$; $RSD_R = 2.34\%$

A. Apparatus

(**a**) *Extraction system.*—Capable of simultaneous extraction of 6 test portions. Extraction unit for solvent addition to cups, 2-stage extraction process, and solvent recovery cycle. Service unit to supply hot oil through insulated tubing to extraction unit and to pump air for evaporation of last traces of solvent from cups (Soxtec System meets these specifications).

(**b**) *Thimbles and stand.*—26 × 60 mm, cellulose thimbles, and stand to hold 6 thimbles.

(**c**) *Extraction cups.*—Al, 44 id, 60 mm height.

(**d**) *Glass beads.*—3–4 mm diameter.

(**e**) *Mechanical convection oven.*—Maintaining 125° ± 1°C.

Items (**a**)–(**c**) are available as Soxtec system from Perstorp Analytical/Tecator, Inc. (2875 C Towerview Rd, Herndon, VA 22071, USA).

B. Reagents

(**a**) *Petroleum ether.*—To meet specifications in **945.16A** (*see* 27.4.04).

(**b**) *Sand.*—<0.004 g extractables/5 g.

(**c**) *Cotton.*—Defatted.

C. Determination

Accurately weigh ca 3 g test portion into thimble. Add sand to test portion and mix with glass rod. Place thimble in thimble stand and dry 1 h in 125°C oven. Remove from oven and let cool. Loosen test portion/sand mixture using glass rod. Wipe glass rod with small amount of cotton and place cotton in top of thimble. Transfer thimble to extraction unit.

Accurately weigh extraction cup containing a few glass beads. Extract thimble with dried mixture with 40 mL petroleum ether in boiling position for 25 min and in rinsing position for 30 min. Adjust temperature of extraction unit to ensure condensation rate ≥5 drops/s. At completion of extraction, close condenser valves and recover ether.

Dry cup and contents 30 min in 125°C oven. Cool and weigh.

D. Calculations

Calculate percent fat in test sample as follows:

$$\text{Fat content, \%} = \frac{(B - C) \times 100}{A}$$

where A = g test portion weight, B = g weight of extraction cup after drying, and C = g weight of extraction cup prior to extraction.

Reference: *J. AOAC Int.* **75**, 289(1992).

AOCS Official Method Ba 6-84

Replaces Ba 6-49 and Ba 6-61 • Reapproved 1997

Crude Fiber

DEFINITION

This method determines, as crude fiber, the loss on incineration of the oven-dried residue remaining after digestion of the sample with dilute sulfuric acid and dilute sodium hydroxide, as specified in the conditions outlined in the procedure.

SCOPE

Applicable to grains, meals, flours, feeds and all fiber-bearing material from which the oil or fat can be extracted leaving a workable residue. This is a joint AOCS–Association of Official Analytical Chemists (AOAC) method (References, 1).

REAGENTS

1. Sulfuric acid solution—containing 1.25 g H_2SO_4 per 100 mL (1.25% or 0.255 N) (see Notes, *Caution*).
2. Sodium hydroxide solution—containing 1.25 g NaOH per 100 mL (1.25% or 0.313 N) (see Notes, *Caution*).
3. Ceramic fiber—Prepare by blending about 150 g of ceramic fiber with 250 mL distilled water in a Waring blender at high speed for 5 min. Store in plastic jar. Individual mats may be prepared by slowly pouring 75–100 mL of mixed slurry into a Buchner funnel (without suction) and allowing it to settle before turning on the vacuum. Optimum mat size for 70-mm funnel is 3–4 g; for 55-mm funnel, 2–2.5 g. Alternate preparation: Stack US no. 6 mesh sieve on top of no. 10 mesh sieve. Place suitable amount of ceramic fiber on no. 6 mesh sieve and rub across sieve until all of the fiber has passed through the openings. Discard small amount left on no. 6 sieve. Shake material on no. 10 mesh sieve for about 1 min, and discard fines that pass through the sieve. Save and store the fiber remaining on the no. 10 mesh sieve. Any method of preparation is acceptable if it results in the making of a satisfactory mat in the Buchner funnel. Discarding the fines will reduce the magnitude of the blank determination.
4. Glass wool—Pyrex™ (Owens–Corning no. 3950), or equivalent.
5. Methyl alcohol 95%, isopropyl alcohol or ethyl alcohol 95%.
6. Diethyl ether—ACS grade, or petroleum ether—AOCS Specification H 2-41 (see Notes, *Caution*).
7. Dow–Corning Antifoam A Emulsion—diluted 1:4 with water. This is also available in a solvent-dilutable form as Antifoam A Compound.
8. Bumping chips or granules—broken alundum crucibles or alundum granules, or equivalent (see Notes, 2).

APPARATUS

1. Crude fiber digestion apparatus—with condenser to fit a 600-mL beaker and a hot plate adjustable to a temperature that will bring 200 mL of distilled water at 25°C to a rolling boil in 15 ± 2 min (suitable equipment is available from Precision Scientific Co., Chicago, IL, USA).
2. 600-mL Beaker—designed to fit digestion apparatus.
3. Ashing dishes—Silica, Vitrosil 70 × 15 mm, porcelain Coors no. 60197, or equivalent.
4. Air oven—maintained at 130 ± 2°C.
5. Electric muffle furnace—with rheostat control and pyrometer that will maintain a temperature of 600 ± 15°C.
6. Desiccator—with efficient desiccant. Calcium chloride is not satisfactory. Drierite 4–8 mesh is satisfactory. See AOCS Specification H 9-87.

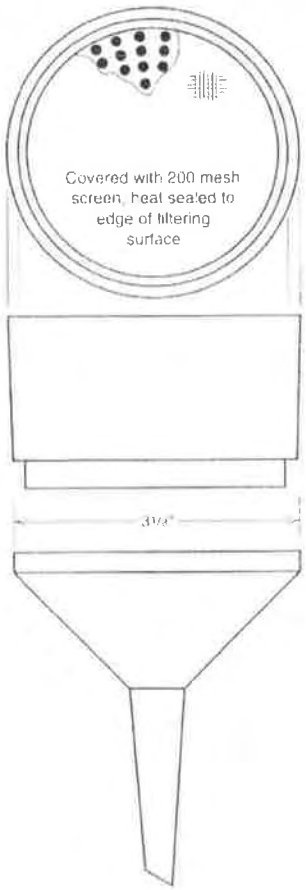

Figure 1. California State modified Buchner funnel, two-piece polyethylene (55- or 70-mm dia).

7 Filtering device—consisting of a no. 200 mesh US series equivalent stainless steel screen and constructed to afford easy and efficient washing of the digested residues (see Fig 1 and Notes, 3 and 4) When extremely fine material is analyzed, it is necessary to precoat this filtering device to avoid loss. In this case, the additional "blank" which may be occasioned by extra ceramic fiber should be determined (see Notes, 5).

8. Suction filtering apparatus—to accommodate the California State modified Buchner funnel. This includes suction flask attached to a trap in line with a water aspirator or other suction line, equipped with a valve to break the suction.

9. Apparatus designed to preheat alkali, acid and wash water to 100°C. Suggested design consists of a tank composed of sheet copper, containing three coils made of 12.5 ft long, ⅜-in. copper tubing. Inlets and outlets are soldered where tubing passes through tank walls. Fill with water and fit with reflux condenser to reduce evaporation. Two 750-watt hot plates will maintain boiling. Use Tygon tubing to connect the coils to reservoirs of distilled water, 1.25% acid and 1.25% alkali, with gum tubing for delivery from the outlets. The capacity is adequate for 60 fiber analyses in 8 hr. See Figure 2.

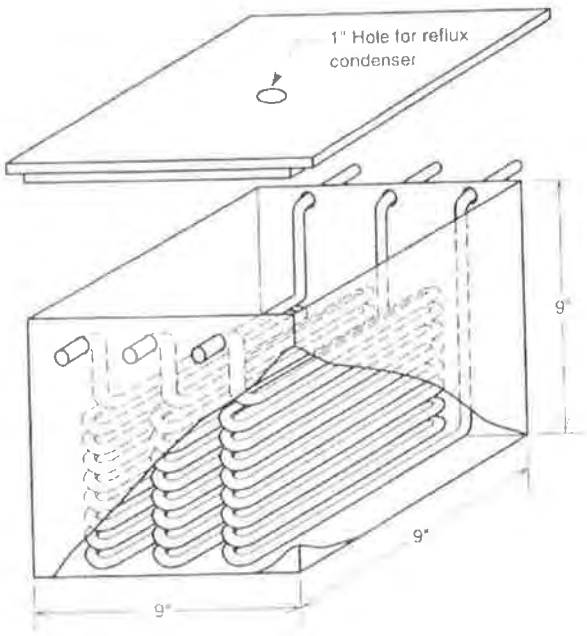

Figure 2. Continuous heater for distilled water, 1.25% alkali and 1.25% acid.

PREPARATION OF SAMPLE

1. Reduce a 1000-g sample using a divider or other suitable means to about 100 g and place in a sealed, moisture-proof container. Determine the moisture immediately.
2. Grind the remainder of the 100-g portion obtained above through a laboratory mill to a uniform fineness.

Note—Laboratory experiments have shown that the Weber mill (screen size .033–.040 in.), the micro screen (1/25–1/16 in.) and the Wiley mill (1-mm screen) will give grinds of comparative fineness. While other mills may be suitable, it is recommended that one of the mills mentioned above be used for preparing the sample for crude fiber determination. Most materials will lose moisture during grinding, and the moisture should be determined on the ground sample as the portion is taken for the crude fiber determination.

PROCEDURE USING CERAMIC FIBER (see Notes, 5)

1. Extract 2 g of the ground material with diethyl or petroleum ether to remove fat. Transfer to a 600-mL beaker, being careful to avoid fiber contamination from paper or brush used in the process.

   Note—If fat content of sample is below 1%, the extraction step can be eliminated.

2. Add about 3 g of prepared ceramic fiber for 70-mm Buchner funnel or about 2 g of prepared ceramic fiber for 55-mm Buchner funnel, 200 mL of boiling H_2SO_4 (0.255 N) and 1 drop of diluted antifoam agent. Add alundum chips or granules to reduce bumping.

   Note—This antifoam agent will give high results if used in excess. Use only when necessary to control foaming.

3. Place the beaker and contents on the digestion apparatus with the hot plate at the temperature specified in Apparatus, 1. Digest at boiling temperature for exactly 30 min, rotating the beaker periodically to keep any solid matter from adhering to the sides.

4. Remove at the end of the 30-min period and filter, using the California State modified Buchner funnel, according to the following procedure.

   (a) With the Buchner funnel mounted on the suction flask and the suction on, pour the entire contents of the digestion beaker into the funnel. Rinse the beaker with 50–75 mL of boiling water and wash through the funnel. Repeat with three more 50-mL washings, and allow the vacuum to dry the funnel.

   (b) Remove the mat and digest by snapping the bottom part of the funnel against the top while covering the stem with the thumb or forefinger and place the mat and digest in the original digestion beaker. Rinse funnel with 200 mL of boiling 1.25% alkali and digest for exactly 30 min at temperature specified in Apparatus, 1.

   (c) At the end of the alkali digestion, immediately filter contents of beaker through a Gooch crucible, equipped with a glass-fiber filter disk to fit over the bottom of crucible or a 5-mm thick mat of ceramic fiber. Rinse beaker twice with 25-mL portions of 0.255 N H_2SO_4. Then rinse beaker with three 50-mL portions of distilled water. Finally, pour 25 mL of alcohol into the crucible and allow the vacuum to dry the filter. Remove crucible.

5. Dry crucible and contents at 130°C for 1 hr. Cool in a desiccator and weigh. Incinerate for 30 min at 600 ± 15°C. Cool in a desiccator and weigh again.

PROCEDURE USING GLASS WOOL (see Notes, 5)

1. Extract 2 g of the ground material with diethyl or petroleum ether to remove fat. Transfer to a 600-mL beaker, being careful to avoid fiber contamination from paper or brush used in the process.

   Note—If fat content of sample is below 1%, the extraction step can be eliminated.
2. Add 200 mL of boiling 1.25% H_2SO_4 and 1 drop of the diluted antifoam agent. Add alundum chips or granules to reduce bumping, if desired.

   Note—This antifoam agent will give high results if used in excess. Use only when necessary to control foaming.
3. Place the beaker and contents on the digestion apparatus with the hot plate at the temperature specified in Apparatus, 1. Digest at boiling temperature for exactly 30 min, rotating the beaker periodically to keep any solid matter from adhering to the sides.
4. Remove at the end of the 30-min period and filter, using the California State modified Buchner funnel, according to the following procedure:
   (a) Prepare the Buchner funnel (55 mm i.d.) by cutting a round piece of glass wool of sufficient size to completely line the bottom and sides of the funnel. The glass wool pad should be shaped to the inside of the funnel. Then apply suction, using a stream of water, to form a seal between funnel and glass wool.
   (b) Care must be taken not to separate the glass fibers in the mat. This is necessary for maximum retention of the sample on the glass wool.
   (c) Glass wool no. 3950 is packed in rolls. Carefully unroll as needed. Each large layer consists of six thin layers. Normal meals, feeds, grains and feed ingredients will be retained by a three-layer lining (see Notes, 6).
5. With the Buchner funnel mounted on the suction flask and suction applied, pour the entire contents of the digestion beaker into the funnel. Rinse the beaker with 50–75 mL of boiling water and wash through the funnel. Repeat with three more 50-mL washings and allow the vacuum to dry the funnel.
6. Transfer the glass fiber mat and digest from the funnel into the original digestion beaker. Using a stream of 1.25% alkali, rinse funnel into beaker. Add additional alkali to bring the volume to 200 mL, and digest for 30 min at the temperature specified in Apparatus, 1. Digest at boiling point for exactly 30 min, rotating the beaker periodically to keep any solid matter from adhering to the sides.

   Note—The glass fiber mat must be immersed in the solution at all times; gentle swirling of the solution at times will prevent entrapment of air and aid in keeping material in contact with the solution.
7. Prepare a glass wool fiber mat about 10-mm thick in the Gooch crucible. Then transfer the glass fiber mat and digest to the Gooch crucible. A convenient way to do this is to pick up the wet fibers using large tweezers and twist the material into a narrow or composite mass, making it possible to place in the crucible without touching the outer edge. Then compress the materials as necessary with glass rod and pour. With the Gooch crucible mounted on a suction flask and suction applied, pour the entire contents of the digestion beaker through the Gooch crucible.
8. Rinse the beaker and glass rod using 25 mL of boiling 1.25% H_2SO_4, followed with three 50-mL washes with hot distilled water. Add all washes to the Gooch crucible. Finally, wash the crucible with 25 mL of alcohol, allow the vacuum to dry the filter mat and remove the crucible.
9. Dry the crucible and contents at 130°C for 1 hr. Cool in a desiccator and weigh. Incinerate for 30 min at 600 ± 15°C. Cool in a desiccator and weigh again.

CALCULATIONS

1. Crude fiber in ground sample, $\% = \dfrac{(A - B) \times 100}{W}$

   Where—

   A = the loss in mass from incineration of sample
   B = the loss in mass from incineration of blank
   W = mass, g of sample

2. Crude fiber, $\% = \dfrac{(100 - \%\ \text{moisture desired})}{(100 - \%\ \text{moisture in ground sample})}$

PRECISION

1. Using ceramic fiber (References, 2)—mean, 14.24; average range, 0.24; average maximum spread, 2.24; pooled standard deviation, 0.62; S_r, 0.28; S_b, 0.55.
2. Using glass wool—
   (a) Within-lab SD: SD = 6% of the mean crude fiber (CF) value, but not less than 0.35% CF.
   (b) Between-lab SD (single determinations): SD = 7% of the mean CF value, but not less than 0.4% CF.
   (c) Between-lab SD (replicate determinations): SD = 5% of the mean CF value, but not less than 0.3% CF.

   Note—The SD varies with the size of the numerical value of the CF, but at low values reaches a minimum. Example: At 10.0% CF the SD for within-lab determinations would be 6% of 10.0, or 0.6%. However, at 2.0% CF the SD would be 0.35%.
3. Using asbestos (see Notes, 7)—Precision of the method was established using AOCS Official Method M 1-59 (declared surplus in 1989), modified by eliminating duplicates. Four preground samples, ranging from 2 to 12% crude fiber were submitted to twelve collaborators; 384 results were obtained and calculated on a 95% confidence limit basis:
   (a) Agreement within laboratories: Two single determinations performed in one laboratory using the Oklahoma Filter Screen shall not differ by more than 0.48%. Two single determinations performed in one laboratory using the California State modified Buchner funnel shall not differ by more than 0.68%.
   (b) Agreement between laboratories: Single determinations performed in two different laboratories using either filtering device shall not differ by more than 0.79%.
   (c) These precision data do not apply to products with high fiber, such as alfalfa meal, or products that present filtering problems, such as yeast.

NOTES

Caution

Sulfuric acid is a strong acid and will cause severe burns. Protective clothing should be worn when working with this acid. It is an oxidizing agent and should not be stored in the vicinity of organic materials. Use great caution in mixing with water due to heat evolution that can cause explosive spattering. Always add the acid to water, never the reverse.

Alkalies can burn skin, eyes and respiratory tract severely. Wear heavy rubber gloves and face shield to protect against concentrated alkali liquids. Use effective fume-removal device or gas mask to protect respiratory tract against alkali dusts or vapors. When working with extremely caustic materials like sodium hydroxide and potassium hydroxide, always add pellets to water and not the reverse. These alkalies are extremely exothermic when mixed with water. Take precautions to contain the caustic solution in the event that the mixing container breaks from the extreme heat generated.

Diethyl ether is highly flammable and is a severe fire and explosion hazard when exposed to heat or flame. It is a central nervous system depressant by inhalation and skin absorption. It will form explosive peroxides upon exposure to light. Handle empty containers, particularly those from which ether has evaporated, with extreme caution. Explosive limits in air are 1.85–48%. The TLV is 400 ppm in air. A fume hood should be used at all times when using diethyl ether.

Petroleum ether is extremely flammable. Avoid static electricity. The explosive limits in air are 1–6%. A fume hood should be used at all times when using petroleum ether.

NUMBERED NOTES

1. This equipment may be available from Porter–Warner, Rock Hill, SC, USA, or E. J. Bartell Co., Renton, WA, USA (the product available from E. J. Bartell Co. has the trade name "Cerafiber").
2. R.R. alundum 90 mesh manufactured by the Norton Co., Worcester, MA, USA.
3. Stainless steel, type 304, is recommended. Available from W. S. Tyler, Inc., Mentor, OH, USA.
4. The two-piece polyethylene funnel is similar to Fisher no. 10-362-B or -C, or Nalge no. 4280. 55 or 70 mm. The funnel is supplied without the 200 mesh screen. This can be sealed easily to the filtering surface of the funnel using a small tip soldering iron.
5. A blank determination must be run using all reagents, but without any sample.
6. Materials that will pass through a 200 mesh sieve will require all six layers of glass wool.
7. Due to the hazard associated with its use, asbestos was eliminated from this method in 1989. The precision data were retained for historical and informational purposes only.

REFERENCES

1. *Official Methods of Analysis*, AOAC International, 16th ed., Gaithersburg, MD, 1995, Chapt. 4, p. 26, Method 962.09.
2. *J. Assoc. Off. Anal. Chem. 65*:265 (1982).

OTHER REFERENCES

J. Assoc. Off. Anal. Chem. 42:222 (1959).
Ibid. 43:335 (1960).
Ibid. 44:567 (1961).
Ibid. 45:578 (1962).

AOCS Official Method Ba 5a-49

Formerly Ba 5-47 • Reapproved 1997

Ash

DEFINITION

This method determines as ash the residue remaining after incineration under the conditions specified for the test.

SCOPE

Applicable to cake and meal from cottonseed, soybeans, peanuts and flaxseed.

APPARATUS

1. Porcelain combustion capsules—Coors no. 170, size no. 3, 25-mL capacity.
2. Electric muffle furnace—with automatic pyrometer control to regulate at a temperature of 600 ± 15°C.
3. Desiccator—containing an efficient desiccant. Calcium chloride is not satisfactory. See AOCS Specification H 9-87.

PREPARATION OF SAMPLE

1. Use sample prepared as directed in AOCS Official Method Ba 3-38, Preparation of Sample.

PROCEDURE

1. Weigh 2 g of well-mixed sample into the previously heated and tared combustion capsule, place in muffle furnace heated to 600°C and maintain at this temperature (± 15°C) for 2 hr.
2. Transfer capsule to a desiccator, cool to room temperature and weigh immediately thereafter.

CALCULATIONS

1. $$\text{Ash, \%} = \frac{\text{mass, g of ash}}{\text{mass, g of sample}} \times 100$$

   Report result to nearest 0.1%.