Basic of Proximate Analysis and New Soxtec 8000 and Hydrocap 8000

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- **Part 1 : Basic of Proximate Analysis**
- Part 2 : New Soxtec 8000 and Hydrocap 8000

Purpose of Proximate Analysis

Estimation and determination of how much of the major food components, which are Moisture, CHO, Lipids, Proteins, Ash, Crude Fibre, exist in a given food. The proximate analyses therefore are:

1. Moisture
2. Crude Fat
3. Crude Protein
4. CHO and Crude Fibre

Total carbohydrate = 100-[moisture + crude fat + crude protein + ash].

Topic

- Sample Preparation
- Crude Protein – Kjeldahl Method
- Fibre
  - Crude Fibre
  - Detergent Fibre
  - Dietary Fibre
- Fat
  - Crude Fat
  - Total Fat

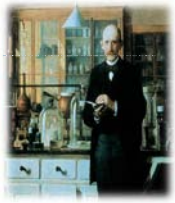
Sample Preparation




Feature	Cemotec™	Cyclotec™	Knifetec™	Homogenizers
Sample Type	Dry samples prior to moisture analysis, up to 15% Moisture and 10% Fat, such as: Grains, Cereals Seeds Beans Dry granular foods Pelleted feed Fertilizer	Dry samples prior to wet chemistry or IR analysis, up to 15% Moisture and 10% Fat, such as: Grains, Cereals Seeds Petfood pellets Hay, straw (dried and cut to 2-3cm) Silage (dried and cut to 2-3 cm) Pelleted feed Leaves Lime, Coal, Tobacco	High-moisture, high-fat and fibrous samples, such as: Grains, Cereals Beans Oilseeds Nuts Peas - wet and dry Maize (Corn) - wet and dry Pelleted feed and Pet food (3-4 mm) Pellets up to 6 mm - with lid for pellets Meat products Vegetables and Fruit	High-moisture, high-fat and fibrous samples, such as: Meat and meat products Whole prepared meals Forage, hay, straw and silage (2097 recommended) Fish* and fish products Vegetables and fruit Chemical and pharmaceutical formulations *May require descaling and or skinning
Sample Size	Up to 14 mm Ø	Up to 10 mm, large inlet up to 40 mm	Maximum 100 ml (50 - 150 g)	(2094): 0,1 - 1,5 kg (2097): 0.1 - 2.5 kg
Grinding Principle	Two discs, one rotating one stationary	Impeller, abrasive ring, and screen	Rotor blade	Various rotor blade

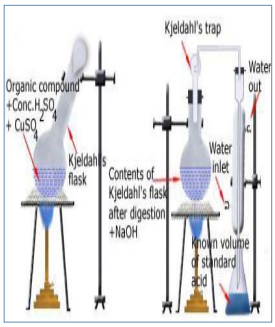
Crude Protein Kjeldahl

Kjeldahl Method



- **Johan Kjeldahl** → Chemist in Copenhagen, Denmark
- **Kjeldahl Procedure**
  - **Digestion** => the reaction between organic compounds and sulfuric acid produced **ammonium salts.**
  - **Distillation** => Ammonium salts reacted with strong alkali. the **ammonia** produced in this step was distilled and dissolved in a standardized solution of hydrochloric acid or sulfuric acid.
  - **Titration** => the solution was back titrated with Sodium hydroxide to indirectly measure nitrogen.
- During the 1880s, ***Kjeldahl used potassium sulfate to raise the boiling point of the acid and mercury as a catalyst to speed the decomposition***. For the back titration process of the released ammonia, he used boric acid buffer solution.

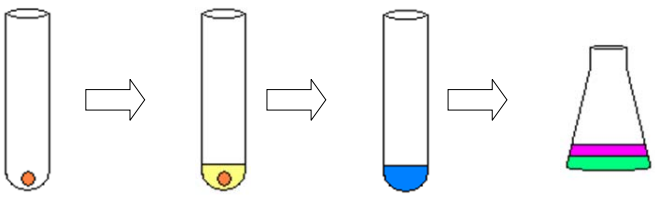
ISO 5983-1 Animal Feeding stuffs-Determination of nitrogen content and calculation of crude protein content Part 1 :



- Wight sample → **0.5-2g**
- Digestion →
  - **Add 15 g K_2SO_4+0.9-1.2g $CuSO_4$**
  - **Add 25 ml H_2SO_4 for 1g sample and 6-12 ml for each additional gram of sample**
  - **Digest 2h**
- Distillation →
  - **Add water 250-350 ml**
  - **100 ml 33% NaOH**
  - **Receiver solution 25 ml**
    - **0.05M,0.125M H_2SO_4**
    - **4% H_3BO_3 solution**
  - **Steam distill until ≥ 150 ml of distillate**
- Titration →
  - **0.05M,0.125M H_2SO_4 → 0.1M,0.25M NaOH**
  - **4% H_3BO_3 solution → 0.05M, 0.125 H_2SO_4**
- Calculation →
  - **Nitrogen Content**
  - **% Protein**

The Kjeldahl Procedure

or



Sample prep. Digestion Distillation Titration&Calculation

Sample preparation of Nitrogen analysis

- **Solid Sample**
  - The particle size should be equal to, or less than, 1 mm
- **Semi Solid Samples**
  - Mortar
- **Liquid Samples**
  - The sample contain precipitates filtration or sedimentation and the filtrate or supernatant liquid taken for analysis
  - Shaking or stirring
- **Sample Weight**
  - Analytical balance accurate to 0.1 mg
  - The actual weight of sample required is dependent on Nitrogen content
    - Homogenous samples (excluding water) 0.1 - 1.0 g
    - Non homogenous samples 1.0 - 3.0 g or more
    - Water samples (dependant on N content) 1.0 - 100 ml

The Digestion Step

Breakage of nitrogen bonds and reduction to Ammonium ions (NH_4^{+})

Organic N + H_2SO_4 $\xrightarrow{K_2SO_4 + Cat.}$ $(NH_4)_2SO_4$ + H_2O + CO_2 + by products

Copper, Selenium, Titanium, Mercury

Catalyst to speed up the destruction

Salt to raise boiling temperature

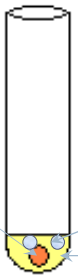
2 Kjeltabs

(~7g K_2SO_4 + catalyst)

Optimal Acid/Salt ratio
12ml / 7g → ratio 1.7

Organic sample

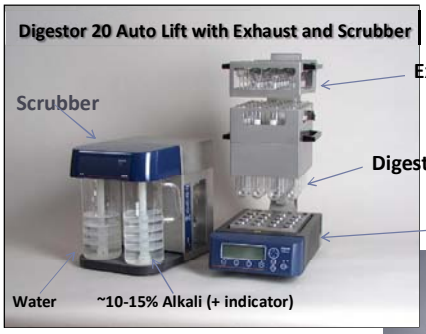
0.5-3g



Boiling **sulphuric acid** (12-15ml)

Temp 420 ºC, Time 1 hr

The Digestion System



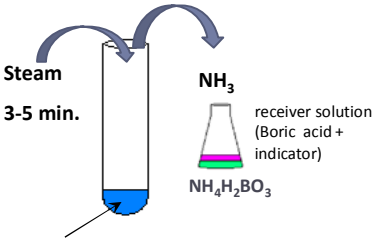
Exhaust

Digestion Tubes

Digestor

The Distillation Step

Separation of ammonia (NH_3)



Digestion mixture
plus
Water 80ml (6:1)
40% **NaOH** 50-60ml (4:1)

$(NH_4)_2SO_4 + 2NaOH \rightarrow 2NH_{3\,(g)} + Na_2SO_4 + 2H_2O$

$2NH_{3\,(g)} + H_3BO_3 \rightarrow NH_4^+ : H_2BO_3^- + H_3BO_3$ (excess)

FOSS-Kjeltec™ 8000 series

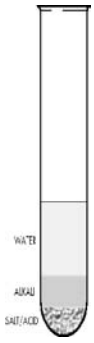

Kjeltec 8420


Kjeltec 8460

Kjeltec Safety – SAfE Patented

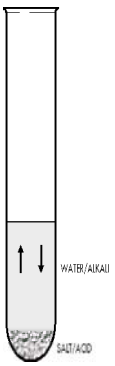
Delay

- Dilution
- Alkali addition
- Delay 2 - 20 seconds to allow for acid/alkali reaction
- Distillation



SAfE (Steam Addition for Equilibration)

- Dilution
- Distillation starts - mixing the acid and water to ensure dilution takes place
- Gradual alkali addition during distillation slowly raises the pH minimizing reaction

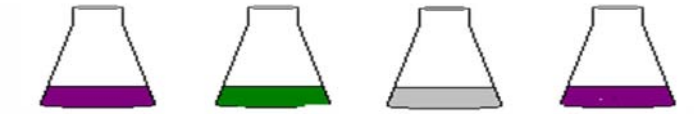


New Global standard
EN ISO 5983-2
2005

Titration

- **Titration with standard acid determines the amount of ammonia and therefore nitrogen in the sample.**
- **Protein can be calculated from a known nitrogen content.**

$NH_4^+:H_2BO_3^- + HCl \longrightarrow NH_4Cl + H_3BO_3$



Initial color | **Plus NH_3** | **Intermediate** | **Final end point**

Receiver Solution
Boric acid + indicators
(Methyl Red &
Bromocresol Green)

Titation with titrant acid
(HCl or H_2SO_4)

Titration & Calculation

Automatic Titrator

Digital Burette

Standard Glassware Burette

$$\% N = \frac{(T - B) \times N \times 14.007}{\text{wt of sample (mg)}} \times 100$$

$$\% \text{ Protein} = \% N \times \text{factor}$$

T = Volume of titrant used for Sample (ml)

B = Volume of titrant used for Blank (ml)

N = Normality of titrant (to 4 decimal places)

14.007 = Molecular weight of Nitrogen

Quality Control – Recovery

Digestion

- Standard substance (Tryptophan, Acetanilide)
- Certified Reference sample
- Internal Reference sample

Distillation

- $(NH_4)_2SO_4$ (purity $\geq$ 99.5%)
- $(NH_4)_2Fe(SO_4)_2.6H_2O$

Trouble Shooting - Problem

☹ **Low/high results**

☹ **Poor Repeatability**

Trouble Shooting

Sample Preparation

- ☑ Balance
- ☑ Sample size
- ☑ Homogeneous

Digestion

Incomplete digestion

- Volume of acid (H_2SO_4),salt (K_2SO_4),catalyst (Cu, Hg, Se, Ti, Cu/Ti)
- Temperature of block
- Digestion Time
- Exhaust head, aspiration rate
- Vacuum level

Trouble Shooting

Digestion

Foaming Sample

- Reduce sample size
- Boiling Rods
- Anti-foam agents → **n-Octanol, H_2O_2**
- Ramp the temperature

Salt cake formation

- Add a small amount (20-30 ml) of water to the sample when cool enough to handle
- Reheat the mixture in a block to "melt" the cake
- Distill the sample with SAfE……

Trouble Shooting

Distillation and Titration

- Alkali volume
- Distillation recovery
  - Dry ammonium sulfate before use → 103-105 °C
  - Purity ≥ 99.5%Titrant concentration
- Burette, air bubbles
- Adsorption of nitrogen in distilling unit (carry over effect), dirty splash head
- An uncontrolled blank contribution, nitrogen in the chemicals/reagents used

- **Acid/Base Mixture Problem**
  - **Distill the sample with SAfE**

Application

DIGESTION SYSTEMS

- Kjeldahl digestion
- Chemical Oxygen Demand (C.O.D.)
- Trace and Heavy metal preparation
- Sample preparation for CFA, FIA etc.

DISTILLATION SYSTEMS

- Kjeldahl Nitrogen/Protein
- Ammonia
- Nitrate
- Nitrite
- Sulphite
- Phenols
- Cyanides
- Volatile Acidity
- etc

Conclusion

Kjeldahl Method
- Digestion
- Distillation
- Titration

→ Nitrogen /Protein 

Trouble shooting

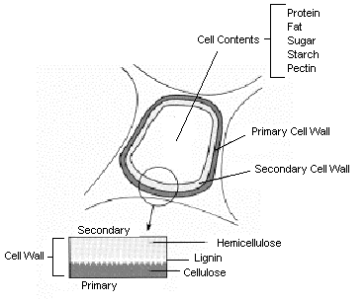
Fibre

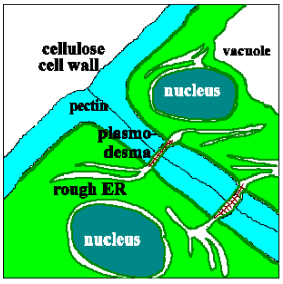
Synopsis

- What is fibre?
- Fibre analysis
  - Crude fibre
  - Detergent fibre
  - Dietary fibre
- Trouble shooting

What is fibre?

Fibre contains cellulose, hemicellulose and lignin + other substances that are difficult or impossible to digest.





Fibre Analysis

- **Crude Fibre - Feed trading**
- **Neutral Detergent Fibre - Optimisation of Energy intake**

→ Fibertec 2010/M6

- **Dietary Fibre – Food labelling**

→ Fibertec E

Sample Preparation – Crude Fibre

- A particle size < 1 mm.
- Homogeneous
- **Fat content**
  - Fat content > 10% → defatted prior to analysis.
  - Fat content is between 1% and 10%
    - ❖ defatting is recommended (ISO5498)
    - ❖should be performed (AOAC, AACC, AOCS)
  - Fat content is less than 1% defatting is not necessary.
- Defat with **Petroleum Ether**
- **High Carbonate**
  - Carbonate (CO_3^{2-}) content is above 1 % carbonate
  - 0.5 M HCl
- **Sample Weight**
  - An analytical balance accurate to 0.1 mg

Manual method

Crude & Detergent fibres

Fibertec 2010

Hot Extraction Unit

Fibertec M6

Cold Extraction Unit

Crucible

Steps in Crude Fibre analysis

AOAC 4.6.01 - Filter Method

- Weigh into beaker
- Defatting if > 1 % fat
- **TRANSFER** to beaker
- Boil with 1.25 % acid
- Filtration and wash
- **TRANSFER** to beaker
- Boil with 1.25 % alkali
- Filtration and wash
- Wash with alcohol
- **TRANSFER** to ashing disc
- Ashing

Fibertec procedure

- Weigh into crucible
- Defatting - Cold Extraction Unit
- Crucible to Fibertec
- Boil with 1.25 % acid
- Filtration and wash
- Boil with 1.25 % alkali
- Filtration and wash
- Wash with acetone
- Ashing

Transfer of Samples Potential Problems

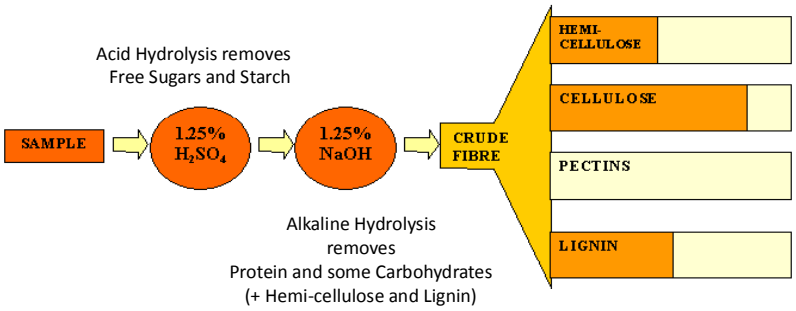
☹ Manual work → Takes time

☹ Loss of sample → Low result

☹ Exposure to reagents → Hazard

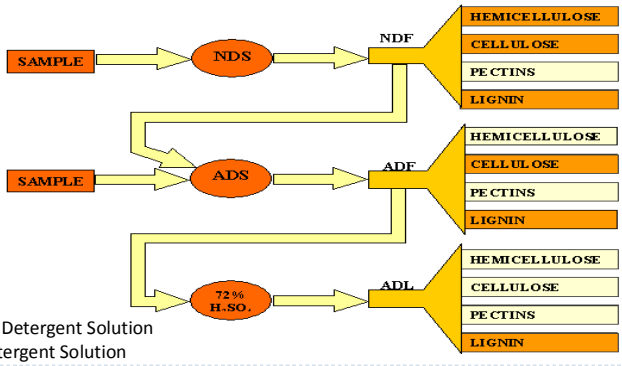
Crude Fibre – Weende Method

DEFINITION
The residue of plant cells after extraction by Acid and Alkaline Hydrolysis



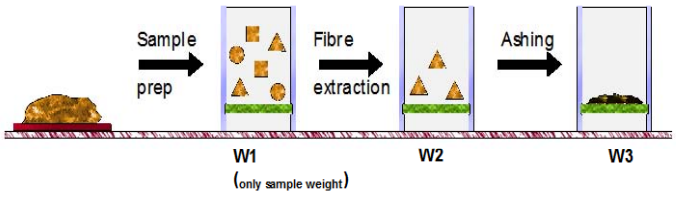
Detergent Fibre – Van Soest Method

DEFINITION
The residue of plant cells
after fractionation using detergent solutions.



NDS = Neutral Detergent Solution
ADS = Acid Detergent Solution

Fibre Calculation



= CH
= Fibre
= Protein

$$\%\,\text{fibre} = \frac{W2-W3}{W1} \times 100$$

Residue Content

- **Crude fibre** — Cellulose 50-80%, Hemicellulose ~20%, Lignin 10-50%
- **Detergent fibre**

Neutral Detergent Fibre (NDF)	Cellulose 100% Hemicellulose 100% Lignin 100%
Acid Detergent Fibre (ADF)	Cellulose 100% Lignin 100%
Acid Detergent Lignin (ADL)	Lignin 100%

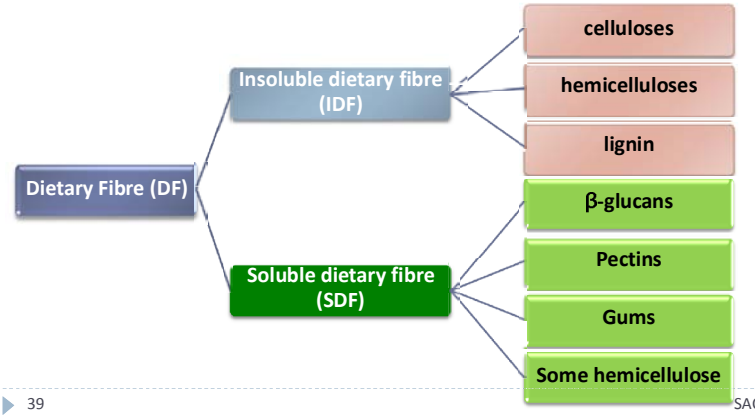
Conclusion – Crude and Detergent Fibre

Method	Principle	Residue content
Crude Fibre (Weende method)	Crude fibre is defined to be the residue after sequential treatment with hot H_2SO_4 (conc. 1,25 %) and hot NaOH (conc. 1,25 %)	Cellulose, 50 - 80% Hemicellulose, approx 20% Lignin, 10 - 50%
NDF (acc. to van Soest)	**N**eutral **D**etergent **F**ibre is defined to be the residue after treatment with a neutral detergent solution (Sodium lauryl sulphate and EDTA)	Cellulose, 100% Hemicellulose, 100% Lignin, 100%
ADF (acc. to van Soest)	**A**cid **D**etergent **F**ibre is defined to be the residue after treatment with an acid detergent solution (Cetyl trimethylammonium bromide in Sulphuric acid solution)	Cellulose, 100% Lignin, 100%
ADL (acc. to van Soest)	**A**cid **D**etergent **L**ignin is defined to be the residue after initial treatment by the ADF method followed by removal of the cellulose fraction through extraction using 72% H_2SO_4	Lignin, 100%

Dietary Fibre

DEFINITION

Dietary fibre is the remnants of plant components that are resistant to hydrolysis by the digestive enzymes of man



Fibertec E

Fibertec E

Sample preparation – Dietary Fibre

- **Solid Sample**
  - particle size 0.3- 0.5 mm

- **Semi solid**
  - Homogenize and dry samples prior to grinding.
  - If sample cannot be heated, freeze-dry before milling. The sample
- **High fat** → fat content > 10 % (recommended (ISO 5498))
  - Defat with 25 ml **Petroleum Ether for** three times
- **High Carbohydrate → Sugar** > 50%
  - Extract three times each with 10 ml of **Ethanol**

Determination of TDF, IDF & SDF

1 g defatted, dried sample

↓

Gelatinization and **Termamyl** incubation (pH 6.0, 30 min, 100°C)

↓

Protease incubation (pH 7.5, 30min, 60°C)

↓

Amyloglucosidase incubation (pH 4-4.6, 30 min, 60°C) → Filtration (Through fritled crucible containing Celite)

↓

Precipitation with 4 volumes ethanol

↓

Filtration

↓

Washing with ethanol and acetone

↓

Drying (TDF) (Correction for undigested protein, ash and blank)

Residue (wash with ethanol and acetone) **(IDF)**

Filtrate → Filtration (add 4 vol of ethanol) → Residue **(SDF)**

TDF = Total Dietary Fibre
IDF = Insoluble Dietary Fibre
SDF = Soluble Dietary Fibre

Trouble Shooting

- **Repeatability**
- **Low/high results**

Trouble Shooting

- Sample Preparation
  - ☺Homogeneous
  - ☺Crude Fiber
    - ☺ Size ≤ 1 mm.
    - ☺ Fat > 1% ,Carbonate >1%
  - ☺Dietary Fiber
    - ☺Size 0.3-0.5 mm
    - ☺Fat > 10% ,Sugar >50%
- Crucible
  - ☺ Clean → Chromic acid, Hydrochloric acid solution (15-20%)

Trouble Shooting

- Celite
  - ☺ accuracy ± 0.1 mg
- Foaming
  - ☺ Antifoam → n-octanol
  - ☺ Turn down heater
  - ☺ Check cooling water
- Wash sample
  - ☺ Hot water

Conclusion

- **Crude Fiber**
  - Fibertec M6, Fibertec 2010
- **Detergent Fiber**
  - Fibertec M6, Fibertec 2010
- **Dietary Fiber**
  - Fibertec E
- **Quality Control**
  - Trouble Shooting

Solvent Extraction Fat

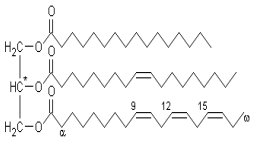
Topic

- Methods for fat determination:
  - Direct Extraction → Crude Fat
  - Total Fat
- Quality Control
  - Trouble Shooting
  - Suggestion

Principle – Fat Analysis

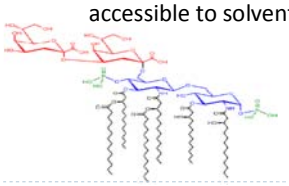
Crude Fat

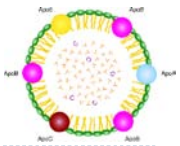
- Crude fat is determined by a solvent extraction.
- non-polar organic solvents such as hexanes, petroleum ether.



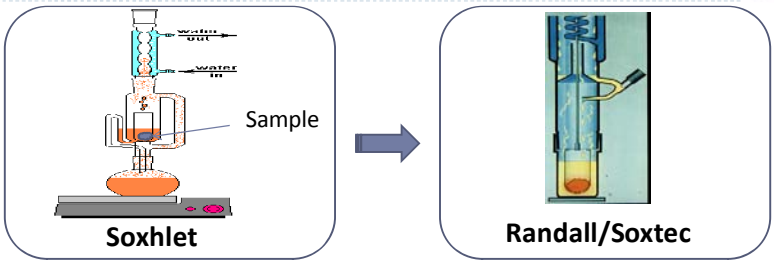
Total Fat

- Total fat determination includes a preparatory acid hydrolysis step and a solvent extraction
- The fat that is bound to other non-solvent solubles as e.g. proteins are separated in hydrolysis step
- Hydrolysis makes chemically or mechanically bound fats accessible to solvent extraction





Sovent Extraction: The Soxtec idea - to improve the Soxhlet idea



Faster	-An automatic four step extraction -Loading/unloading of six samples simultaneously
Cheaper	-Less solvent use -Solvent recovery -Batch operation
Safer	-Explosion proof design -Less exposure to solvent vapours

Sample Preparation

- **Solid Sample**
  - The particle size should be equal to, or less than, 1 mm
- **Semi Solid Samples**
  - Homoginizing == > Mortar
- **Liquid Samples**
  - Samples containing particles, like wastewater, are filtered to collect the parts that will be extracted
  - In samples where it is a suspension, handling including adsorption on inert material such as Celite 566 could be the

Fat Content	Recommended Sample Weight
0-10%	1.5 – 2g ± 0.1 mg
10 – 20%	1 – 1.5g ± 0.1 mg
>20%	1g ± 0.1 mg

Crude Fat and Total Fat Analysis

Crude Fat

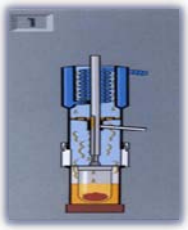
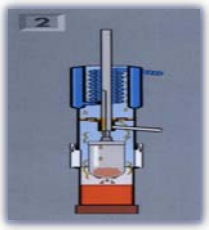
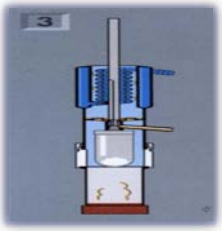
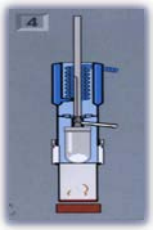
- **Sample Preparation**
- **Solvent Extraction** - Soxtec

Total Fat

- **Sample Preparation**
- **Hydrolysis** – 3-6N HCl

- **Solvent Extraction** - Soxtec

Soxtec Principle

			
Boiling Step ▪ Sample is immersed in boiling solvent ▪Provides rapid extraction of soluble materials ▪For most application, the boiling step is 15 to 25 minutes	**Rinsing Step** ▪Sample is raised out of the boiling solvent ▪Condensed solvent drips through sample and rinses out residuals ▪Usually 30 to 40 minutes	**Solvent Recovery Step** ▪Condensed solvent is collected in the collection vessel ▪This step concentrates the extracted material in the extraction cup and saves solvent for reuse ▪Usually 10 minutes	**Pre-drying Step** ▪Shortened drying time through pre-drying in instrument

Soxtec System

Soxtec 8000

NEW

Hydrotec 8000

Soxtec 2055

Soxtec 2043

Soxcap 2047

Soxtec 2045

Quality Control

☹ **Low/high results**

☹ **Repeatibility**

Trouble Shooting

☺ **Check sample size**
- Size ≤ 1 mm.

☺ **Homogeneous**
- Celite

☺ **Sample weight**
- Accuracy ± 0.1 mg
- Negative weight

☺ **Cup temperature**
- Adjust level between cup and heater

☺ **Drop rate**
- 3-5 drops/s

Recommendation

Before start…..

Check
- ☑ Instrument
  - ➢ Soxtec
  - ➢ Cooling
- ☑ Apparatus
  - ➢ Cup
  - ➢ Thimble

Sample Preparation
- ☑ After Hydrolysis → Dry sample

Solvent
- ☑ AR Grade

Application

Soxtec	Soxtec + Hydrotec/Soxcap
Sample ➢ Soil, wastewater and sludge. ➢ Plastics, petrochemicals, paper, textiles and a very wide rang of other industrial matric **Parameters** ➢ Extractable Matter – Material soluble in a given solvent or range of solvent	**Sample** ▸ Raw Materials, Intermediates and Finished Products in Food, Animal Feed and Petfood **Parameters** ▸ Total Fat (Free and Bound Fat)

Part 2 : New Soxtec 8000 and Hydrocap 8000

Soxtec System

FOSS

Soxtec 2050,2055,2043,2045
&
Soxcap 2047

The new Soxtec 8000
&
Hydrotec 8000

Product Intro
Reinventing Fat Analysis – 3 new products

FOSS

Hydrotec™ 8000

Hydrocap

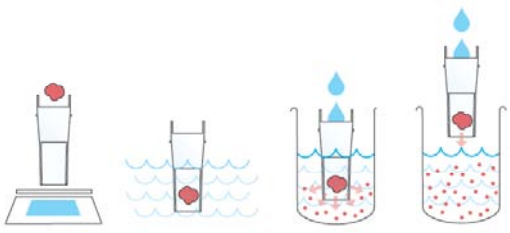
Soxtec™ 8000

Hydrocap	Patented unique Hydrocap single use filter system
Hydrotec 8000	New fully automated hydrolysis (12 pos.)
Soxtec 8000	New fully automated expandable extraction unit (6+6 pos.)

First fully automated Soxhlet system

FOSS

- Patented Hydrocap system for integrated total fat analysis
- Solvent dial for enclosed solvent dosage
- Intelligent water cooling,pivoting hotplates



HydroCap contains sample all the way through

Improving throughput at a lower cost

FOSS

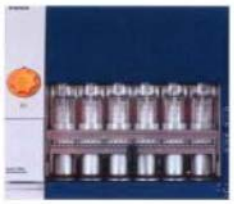
- Save operator time by avoiding filter transfer in total fat
- Flexible extraction according to demand (84 sample/day)
- Simplified operation reduces risk of errors and associated costs

- *Avoid sample handling*
- *Save water*
- *Save solvent*
- *Improve throughput*

Reducing risk in fat analysis

FOSS

- Automation reduces risk of contact with chemicals and solvents
- Technology simplifies operation and reduces the risk of accidents
- Safe system allows broad range of solvents



- No acid handling
- Automated acid collection

- Safe External control

- Speed dial for closed solvent dosage
- Effective cooling to avoid evaporation
- Solvent recovery with leakage sensors

Soxtec 8000

FOSS

- Easy to install (optional customer installation)
- Individual hotplates per cup/sample (run less than 6 sample&no cup adjustment after docking)
- No magnets or thimble adapters made of metal
- Control Unit manage one/two Extraction units (6 or 12 positions system)
- Improved capacity:
  - 7 batches allows for 42 analyses/day or 84/day
  - one batch overnight
- Improved user Interface with big display and graphics
- Solvent leakage sensor and solvent sensor in tank to prevent star of analysis if tank is not emptied
- Improved handing of solvent drainage (flask design and drainage system)
- Backward compatibility – Soxcap 2047 or Hydrolysis Unit 1047

Hydrotec 8000 & Hydrocap

FOSS

Hydrotec 8000

- Automated hydrolysis
  - Acid filling, boiling, cooling, rinsing, draining
- Closed hydrolysis system
  - eliminates emission of acidic corrosive fumes
- Batch handing – with up to 12 samples at a time
- 9 user defined programs

Hydrocap

- Unique Hydrocap single use filter system
- Same sample holder all the way
  - from weighing to extraction
- Hydrocap fits into any Soxhlet system

How to do total fat analysis with the Soxtec 8000 system

FOSS