Nutrient Test Methods (Part 1)

Seminar on Food Nutrition Labelling Test Method

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Outline

- Definition of the parameter
- Some examples of relevant standards or official methods
- Flow Chart for analysis
- Point to notes for some critical steps

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References

- **Method Guidance Notes on Nutrition Labelling and Nutrition Claims, CFS**
- ***Official Methods of Analysis of AOAC International*, 18th edition, 2005, Current Through Revision 2, 2007**
- **GB Standards, ISO Standards and BS Standards**
- **Food analysis: general techniques, additives, contaminants and composition, *Manuals of Food Quality Control*, *FAO Food and Nutrition Paper 14/7*, 1986**

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Core Nutrients (1 + 7)

- Energy
- Total fat
- Protein
- Carbohydrates (Available)
- Sugars
- Sodium
- Saturated fatty acids
- Trans fatty acids

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Other Nutrients

- Dietary Fibre
- Cholesterol
- Unsaturated fatty acids
- Minerals
- Vitamins
- etc.

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Analysis of Energy

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Energy

- **By calculation**

kcal [kJ] per 100g of food **=**

4 [17] x **available carbohydrates** + **4 [17]** x **protein** + **9 [37]** x **total fat** + **7 [29]** x **ethanol** + **3 [13]** x **organic acids**

Note: all parameters are in %(w/w)

Reference: Codex Guidelines on Nutrition Labelling

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Energy - Points to note

- Needs for testing of ethanol or organic acids depends on the ingredients and their levels in the food samples

  e.g vinegar has to test for organic acids

- Combustion method (Calorimeter) is not acceptable

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Energy –
Available proficiency test

- AOAC

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Analysis of Total Fat

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Total Fat (1)

- Refers to the sum of triglycerides, phospholipids, wax ester, sterols and minor amount of non-fatty materials

- Gravimetric methods
- Sum of individual triglyceride X

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Total Fat (2)

- **Examples of AOAC official methods**
  AOAC 991.36 for meat products
  AOAC 948.22 for nut products
  AOAC 948.15 for seafood
  AOAC 922.06 for flour
  AOAC 989.05 for milk

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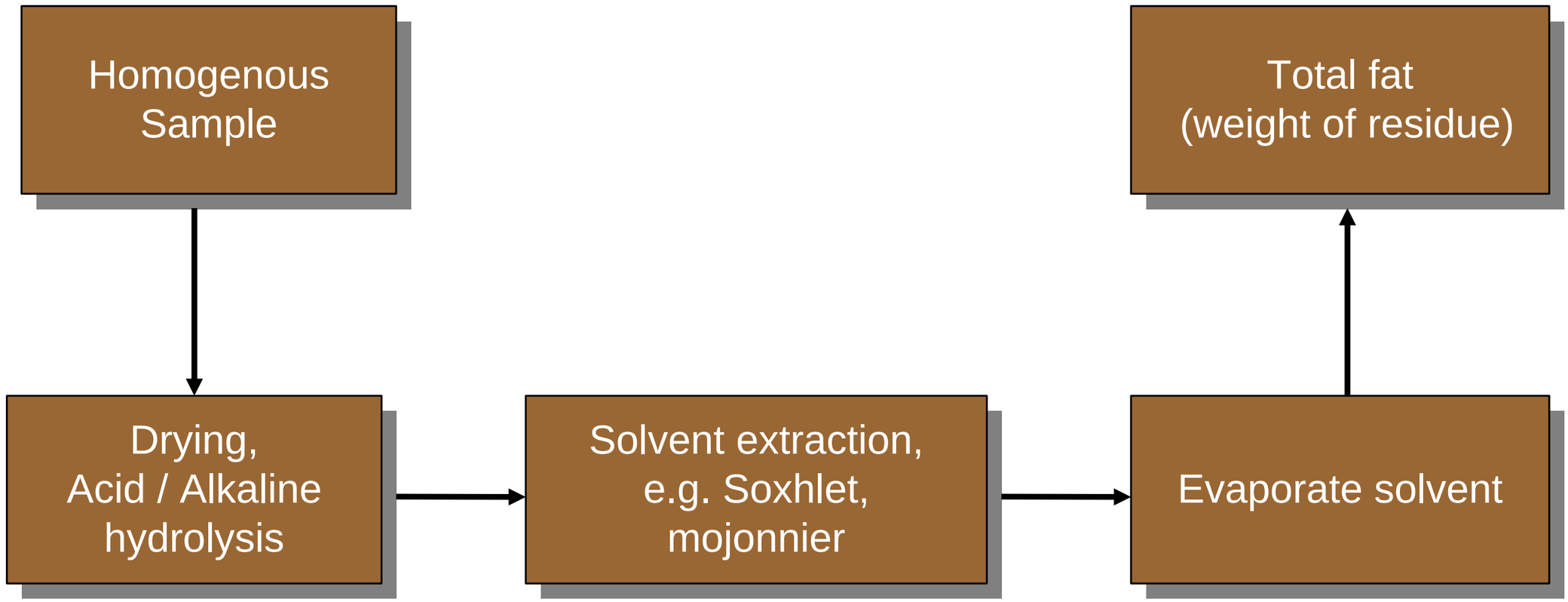
Total Fat (3)

- **Analysis**



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Total Fat (4)

- **Sample preparation**

  representative and homogenous sample

  sample size

  => Definition of “0” ≤ 0.5 g/100g

  e.g. if sample contains 0.5% fat, 1 g sample contains (1 x 0.5%) = 0.005 g of fat

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Total Fat (5)

- Apparatus for blending samples

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Total Fat (6)

- Freeze-dryer

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Total Fat (7)

- **Extraction method depends on food matrix**

e.g. Milk products – alkaline hydrolysis

Flour – acid hydrolysis

Seafood – acid hydrolysis,

solvent extraction

Meat – Soxhlet extraction

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Total Fat (8)

- Acid-hydrolysis

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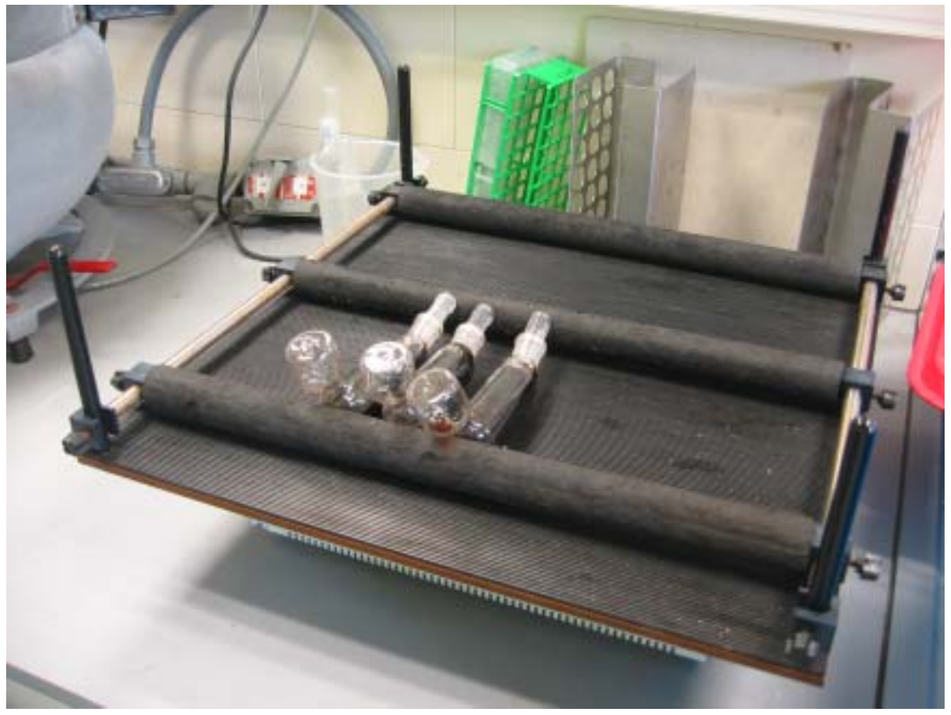
Total Fat (9)

- Extraction



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Total Fat (10)

- **Determine the weight of residue**

  Drying to constant weight

  Prolong heating may increase weight of fat, due to oxidation

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Total Fat - Points to note

- Appropriate method is important
- Acid hydrolysis can produce higher results for cereal products

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Total Fat – Available proficiency test

- **FAPAS**
- **AOAC**
- **LGC**
- **AOCS**

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Analysis of Protein

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Protein (1)

- **Protein = Total Kjeldahl Nitrogen x CF**

  CF = 6.25 for mixed food

- **Examples of AOAC official methods**

  AOAC 928.08 for meat

  AOAC 991.20 for milk

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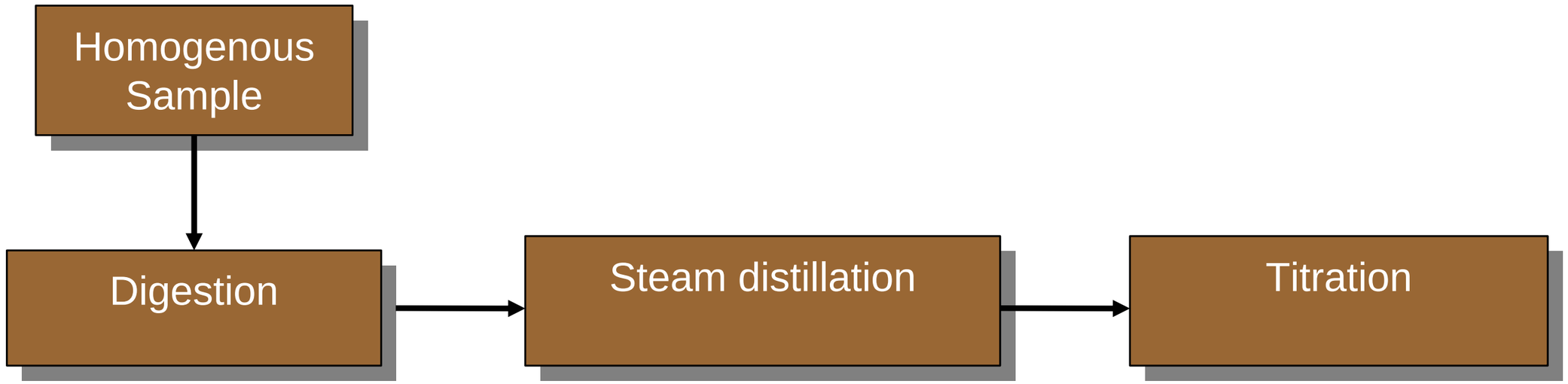
Protein (2)

Analysis (TKN)



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Protein (3)

- **Sample preparation**
  representative and homogenous sample
  sample size
  => Definition of “0” ≤ 0.5 g/100g
  e.g. if sample contains 0.5% protein and the conversion factor = 6.25, 1 g sample contains (1 x 0.5%)/6.25 = 0.0008 g of N (~0.06 mMole) (0.6 ml x 0.1M HCl)

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Protein (4)

- **Digestion**

  Reagents: conc. H_2SO_4, Na_2SO_4 or K_2SO_4 and catalyst (e.g. $CuSO_4.5H_2O$, TiO_2)

  H_2SO_4 : Na_2SO_4 2:1 (initial) ➔ 1:1 (final)

  Temperature: ~420 °C

  Time: ~2 hr

  Appearance of final solution: clear solution

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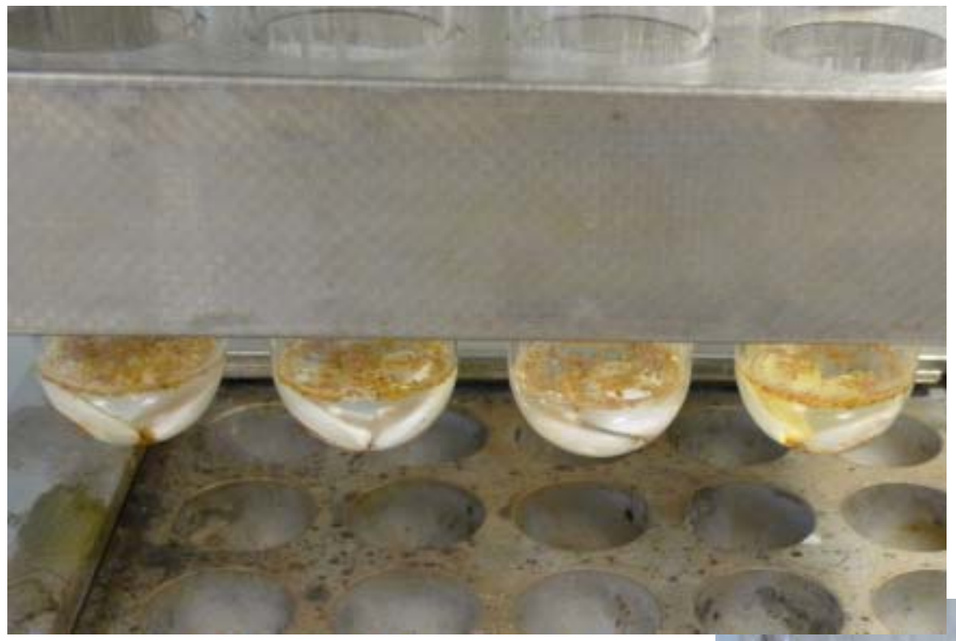
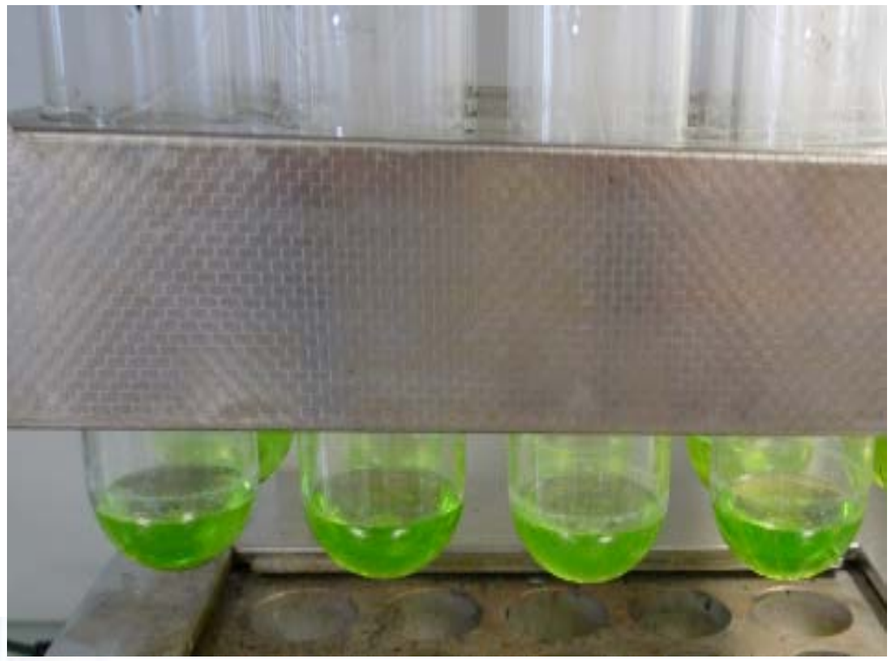
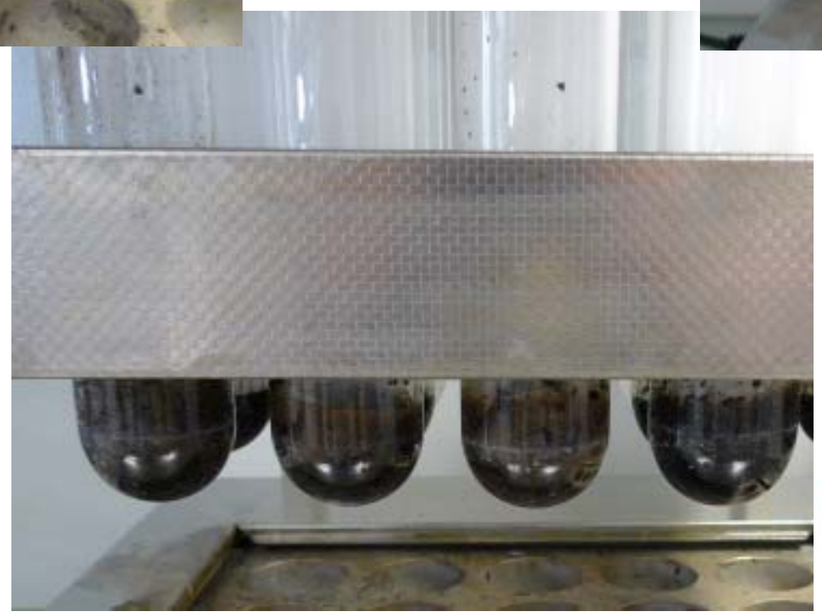
Protein (5)

- Digestion







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Protein (6)

- **Distillation**

  Add NaOH → strongly alkaline

  Distill NH_3 into 1) standardized HCl or 2) boric acid

- **Titration**

  1) with NaOH

  2) with HCl

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Protein (7)

- Distillation & titration

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Protein (8)

- **Analysis by Combustion**

  results similar to TKN

- **Examples**

  AOAC 992.15 for meat and meat product

  AOAC 992.23 for cereal grains

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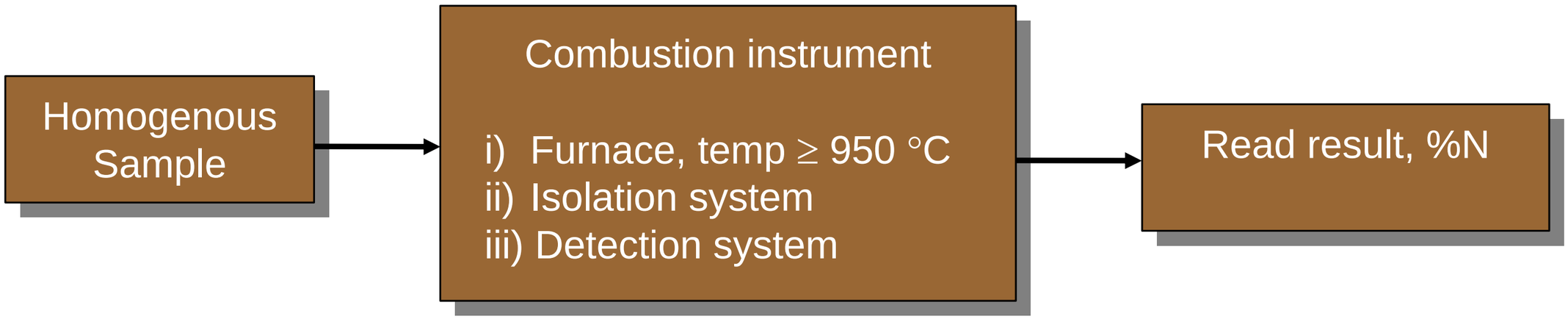
Protein (9)

- **Analysis (Combustion)**



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Protein (10)

- **Sample preparation**

  representative and homogenous sample

  sample size → ≥ 200 mg

- **Instrument**

  Working range

  e.g. 0.5% to 60% protein => 0.08% to 10% N

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Protein (11)

- Combustion Instrument

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Protein (12)

- Analysis by sum of amino acids after hydrolysis of proteins

- may involve huge amount of validation work

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Protein - Points to note

- Conversion factor can vary from 5.18 (almonds) to 6.38 (milk and dairy products)
- Appropriate conversion factor is required
- Check whether a Codex standard is available for the food sample

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Protein –
Available proficiency test

- **FAPAS**
- **AOAC**
- **LGC**

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Analysis of Ethanol

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Ethanol

- **Gas Chromatographic method**
- **Examples**

  AOAC 986.12 for canned salmon

  AOAC 984.14 for beer

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Analysis of Organic Acids

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Organic acid (1)

- Liquid Chromatographic method
- Examples

  AOAC 986.13 for cranberry juice cocktail and apple juice

  GB 5009.157 for foods

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Organic acid (2)

- **Scope of AOAC 986.13**

  quinic acid, malic acid, citric acid

- **Scope of GB 5009.157**

  tartaric acid (酒石酸), butanedioic acid (丁二酸), malic acid (苹果酸), citric acid (柠檬酸)

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Analysis of Carbohydrates

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Carbohydrates (1)

- **Total Carbohydrates =**

  Available carbohydrates + dietary fibre

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Carbohydrates (2)

- **Available Carbohydrates**

  Calculated by Difference :

  100 – (protein + fat + water + ash + ethanol + dietary fibre)%(w/w)

  Direct analysis

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Carbohydrates - Points to note

- **Includes sugar alcohols**

  => use same conversion factor as carbohydrates for energy calculation

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Carbohydrates – Available proficiency test

- AOAC

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Analysis of Water (moisture)

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Water (1)

- **One of major constituents in food**

  as solvent or dispersion medium;

  as in capillaries held by molecular forces;

  as water of hydration held by hydrogen bonding with protein and polysaccharide molecules

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Water (2)

- Analysis - Air oven method
- Examples

  AOAC 935.29 for malt

  AOAC 950.46 for meat

  ISO 1442:1997 / BS 4401-3:1997 for meat and meat products

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Water (3)

- **Analysis - Vacuum Oven Method**

  For high fat and/or high sugar contents

- **Examples**

  AOAC 925.45 for sugars and sugar products

  AOAC 926.12 for oils and fats

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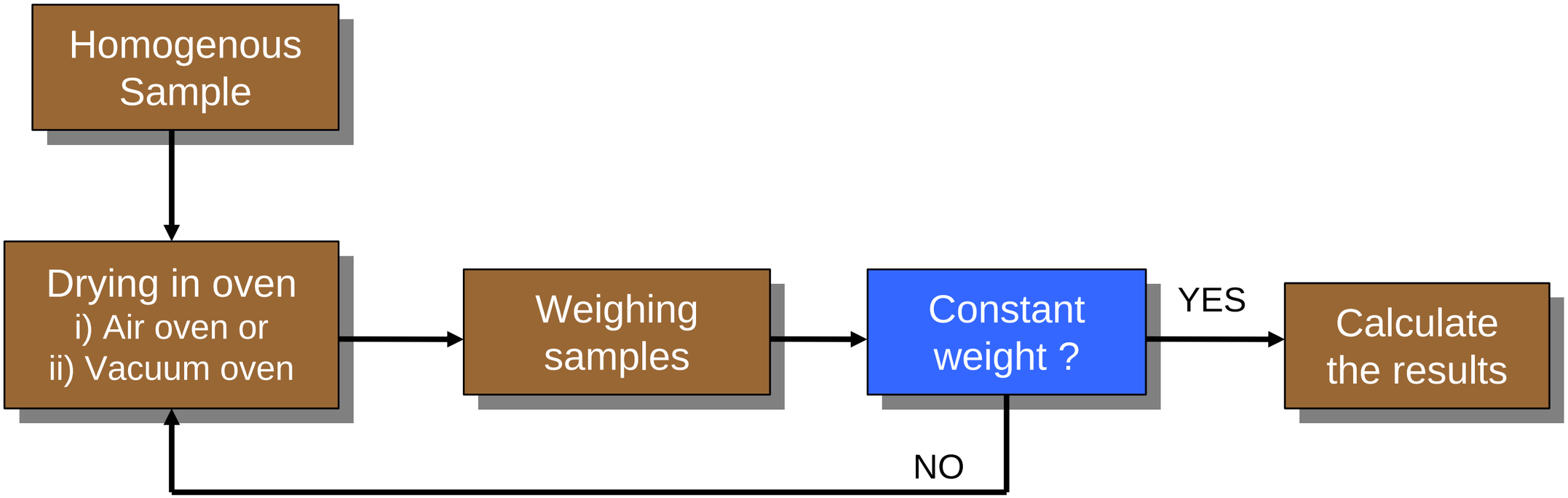
Water (4)

- **Analysis for air or vacuum oven method**



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Water (5)

- **Sample preparation**

  representative and homogenous sample

  sample size

  => accuracy to 0.1 g/100g

  e.g. if sample contains 0.1% water, 1 g sample contains (1 x 0.1%) = 0.001 g (1 mg) of water

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Water (6)

- Sample preparation

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Water (7)

- **Oven conditions**

  Air Oven method:

  temp: depends on the method, usually about 100 °C

  pressure: atmospheric pressure

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Water (8)

- **Oven conditions**

  Vacuum Oven method:

  temp: depends on the method, usually lower than 100 °C, e.g 60 or 70 °C

  pressure: depends on the method, usually < 100 mm Hg

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Water (9)

- **Drying time**

  Depends on the temperature used

  ~ 4 – 18 hours

too long heating time ==> the weight increase due to oxidation

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Water (10)

- **Achieving constant weight**

  Cool the sample to room temperature in desiccator for about an hour

Successive weightings differ only a small amount, e.g. 0.5 mg, 1 mg, 2 mg or 5 mg

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Water (11)

- Desiccator

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Water (12)

- **Analysis – Co-Distillation Method**

  For containing significant amount of volatile substance other than water

- **Examples**

  AOAC 969.19 for chesses

  AOAC 986.21 for spices

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Water –
Available proficiency test

- **FAPAS**
- **AOAC**
- **LGC**

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Analysis of Ash

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Ash (1)

- Inorganic residue after the organic carbonaceous portion and other volatiles have been oxidized and evaporated away

- Examples

  AOAC 945.46 for milk

  AOAC 923.03 for baked products

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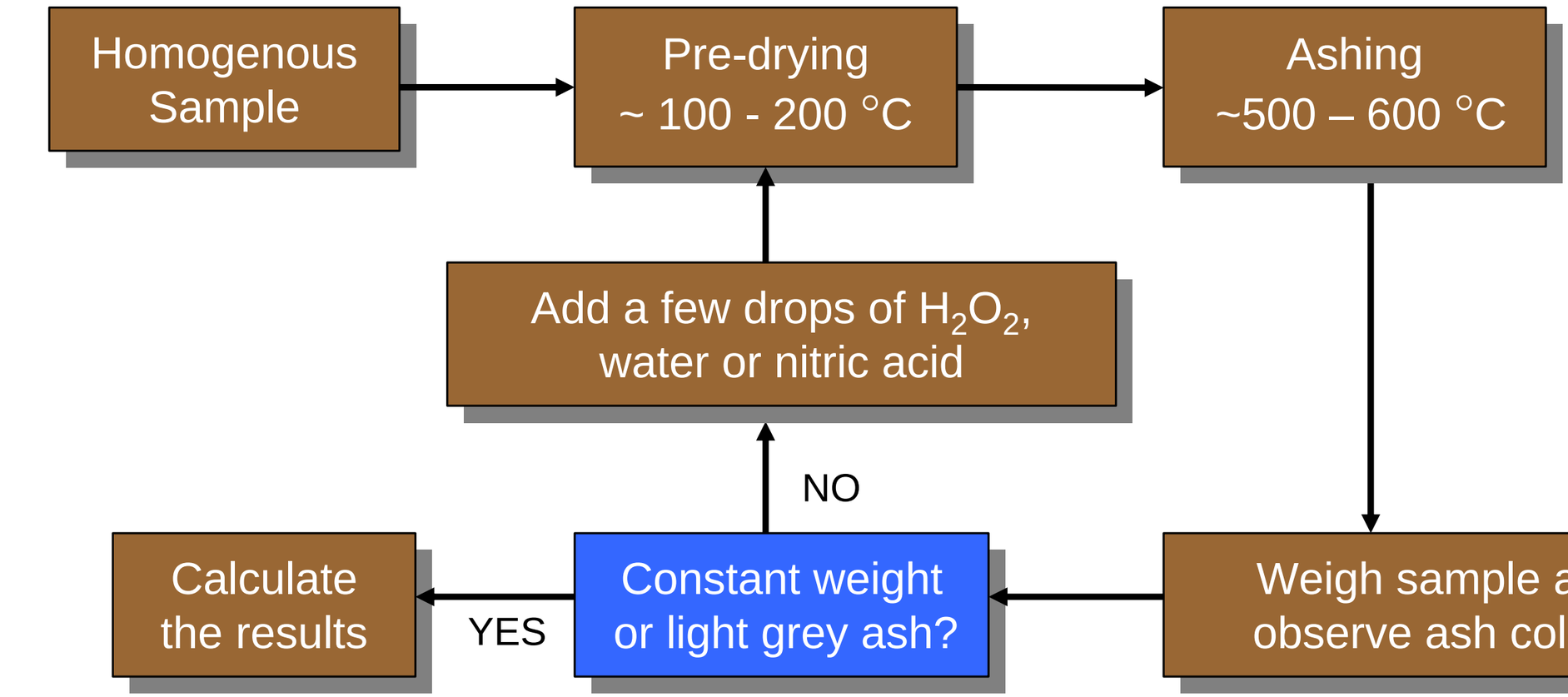
Ash (2)

Analysis



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Ash (3)

- **Sample preparation**

  representative and homogenous sample

  sample size

  => accuracy to 0.1 g/100g

  e.g. if sample contains 0.1% ash, 1 g sample contains (1 x 0.1%) = 0.001 g (1 mg) of ash

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Ash (4)

- **Pre-drying and ashing**

  May combine into one step if the temperature of furnace can be programmed

Avoid splitting and ignition

For high fat food, smoke off without ignition by burner before ashing in furnace

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Ash (5)

- **Results**

  White or light grey ash => no carbon remains

Blank may be required for correction

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Ash (6)

- Weighing and ashing

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Ash (7)

- **Thermogravimetric analyzer**

  Obtain moisture and ash results automatically

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Ash (8)

- Thermogravimetric analyzer

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Ash –
Available proficiency test

- FAPAS
- AOAC
- LGC

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Analysis of Dietary Fibre

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Dietary Fibre (1)

- **HK Regulation - Any fibre analyzed by means of any official methods adopted by AOAC International**

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Dietary Fibre (2)

- **Examples of AOAC official methods**

  AOAC 985.29 Total Dietary Fiber in Foods (insoluble fibre + soluble fibre)

  AOAC 2001.03 (insoluble fibre + high MW soluble fibre + low MW soluble fibre)

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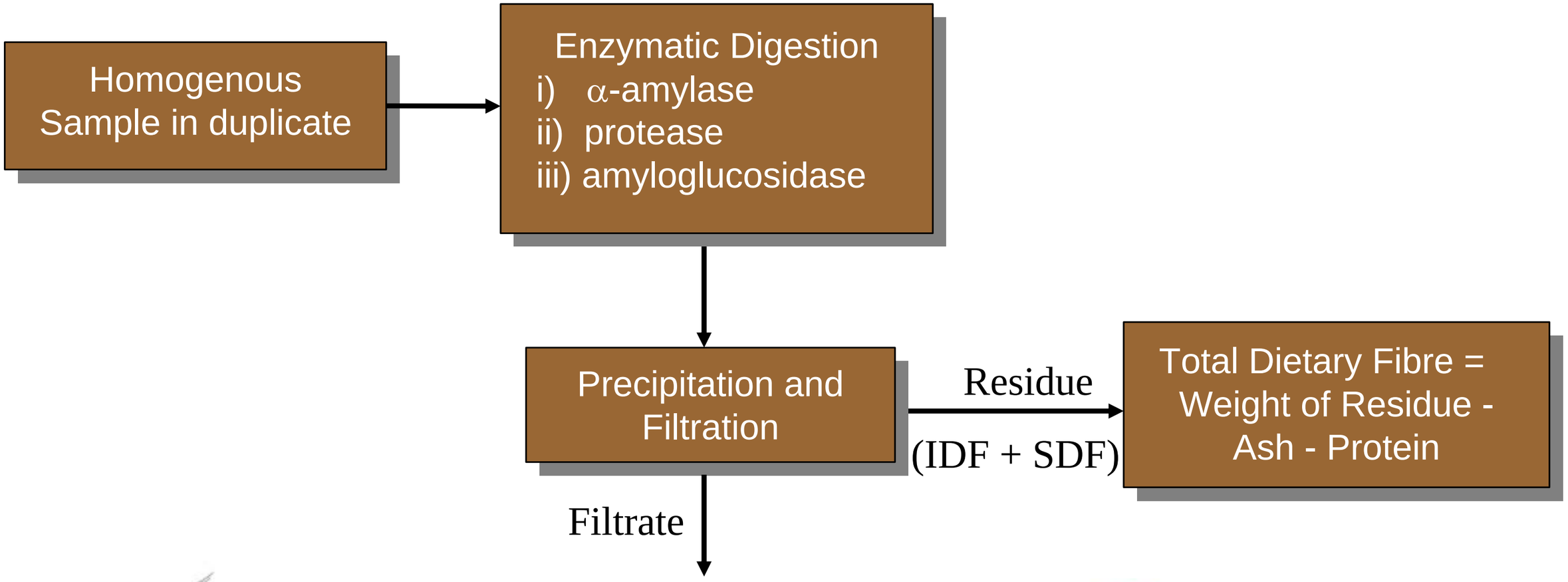
Dietary Fibre (3)

Analysis (AOAC 985.29)



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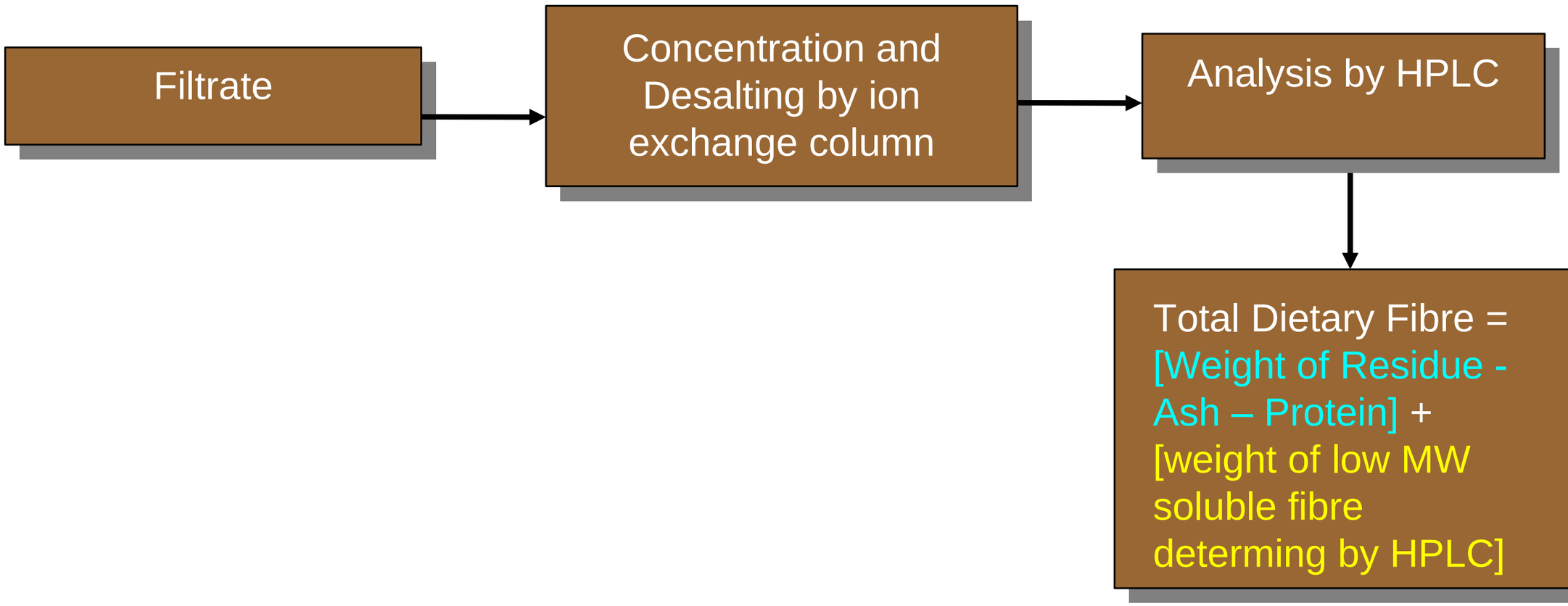
Dietary Fibre (4)

Analysis (AOAC 2001.03)



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Dietary Fibre (5)

- **Empirical Methods**

Results are valid only if the procedure is strictly followed.

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Dietary Fibre (6)

Enzyme purity

Test sample	Activity tested	Sample weight, g	Expected recovery, %
Citrus pectin	Pectinase	0.1	95-100
Stractan (larch gum)	Hemicellulase	0.1	95-100
Wheat Starch	Amylase	1.0	0-1
Corn Starch	Amylase	1.0	0-2
Casein	Protease	0.3	0-2
β-Glucan (barley gum)	β-Glucanase	0.1	95-100

Dietary Fibre (7)

- **Sample preparation**

  homogenous and dried sample, freeze-dry is recommended

defat with petroleum ether if >10% fat content, otherwise false high results

weigh duplicate test portions, differ < 20 mg

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Dietary Fibre (8)

- **Enzymatic digestion**

  i) α-amylase, pH 6.2, 95 – 100 °C for 15 - 30 min

  ii) protease, pH 7.5, 60 °C for 30 min

  iii) amyloglucosidase, pH 4.3, 60 °C for 30 min

Final solution volume ~ 70 ml

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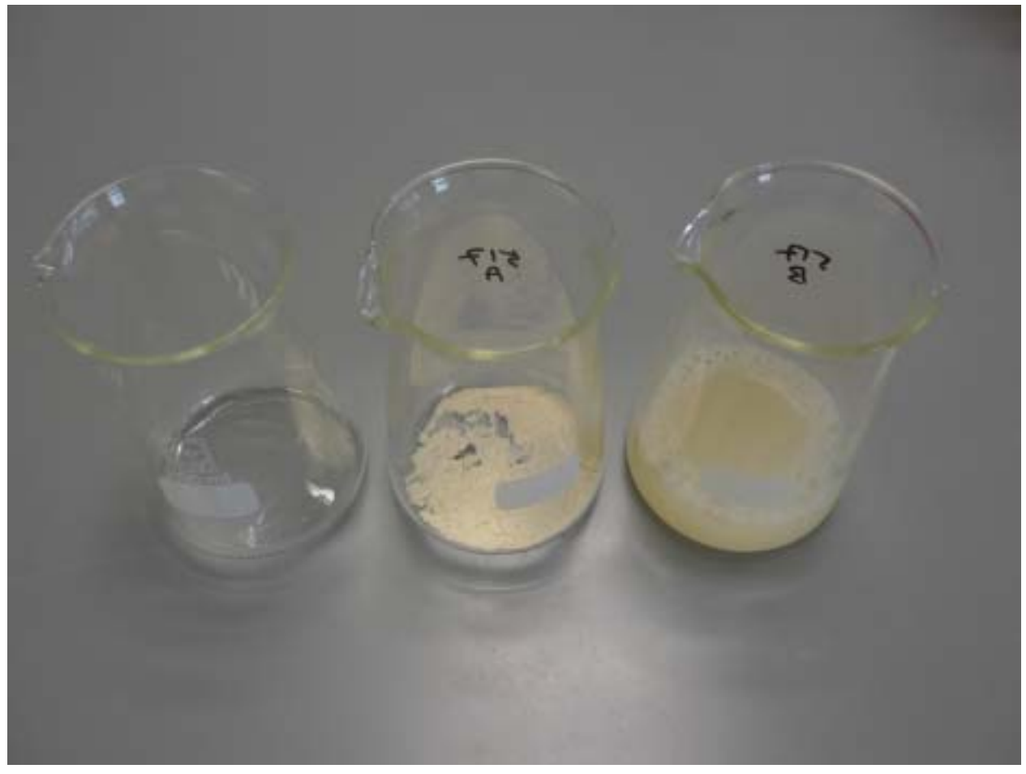
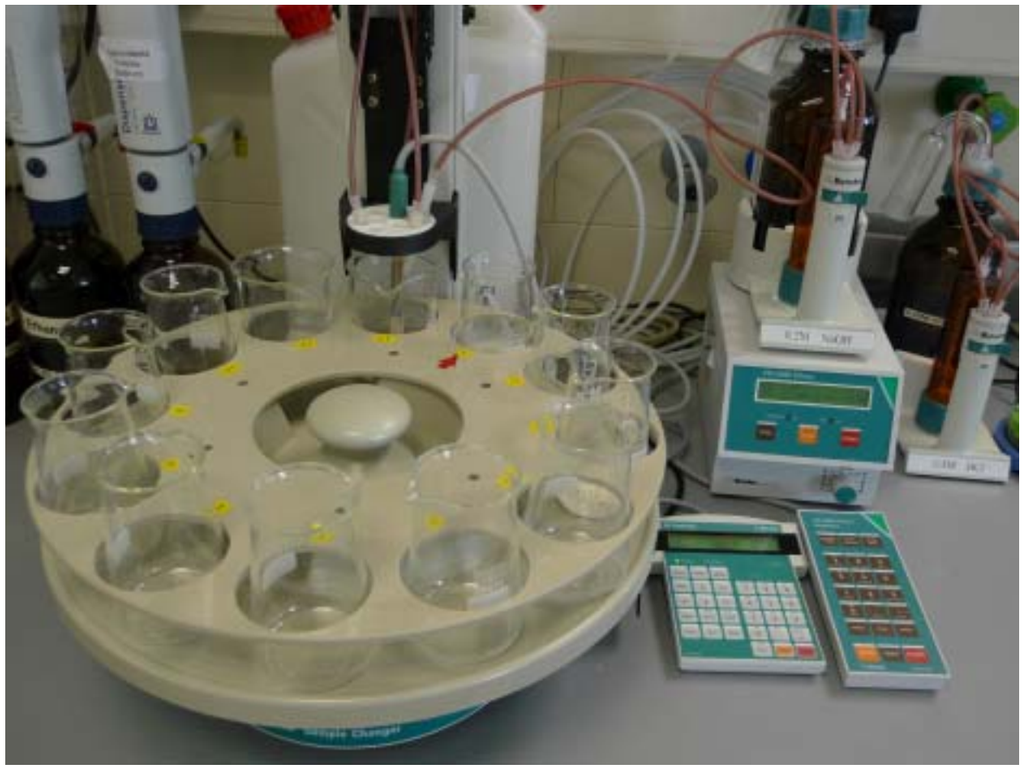
Dietary Fibre (9)

- Preparing for digestion





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Dietary Fibre (10)

- Enzymatic digestion

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Dietary Fibre (11)

- **Precipitation (for soluble fibre)**

  Four volumes of 95% ethyl alcohol

  => 280 ml at 60 °C

let precipitate at room temperature

60 min for AOAC 985.29

overnight for AOAC 2001.03

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Dietary Fibre (12)

- Precipitation

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Dietary Fibre (13)

- **Filtration (by suction)**

  collect the residues (soluble fibre + insoluble fibre) in pre-weight crucibles

ensure quantitative transfer of residues

may take 0.1 to 6 hrs per sample

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Dietary Fibre (14)

- Filtration

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Dietary Fibre (15)

- Residues collected

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Dietary Fibre (16)

- **Results (AOAC 985.29)**

  1 test portion → analyze for protein (CF=6.25)

  1 test portion → analyze for ash (5 h at 525 °C)

TDF = weight of dried residue – ash – protein (IDF + HMWRMD)

Blank value correction

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Dietary Fibre (17)

- **Determination of low low MW resistant maltodextrin (LMWRMD):**

  concentrate the filtrate

  remove salt from buffer by ion-exchange column

  analyze by HPLC with RI detector => LMWRMD

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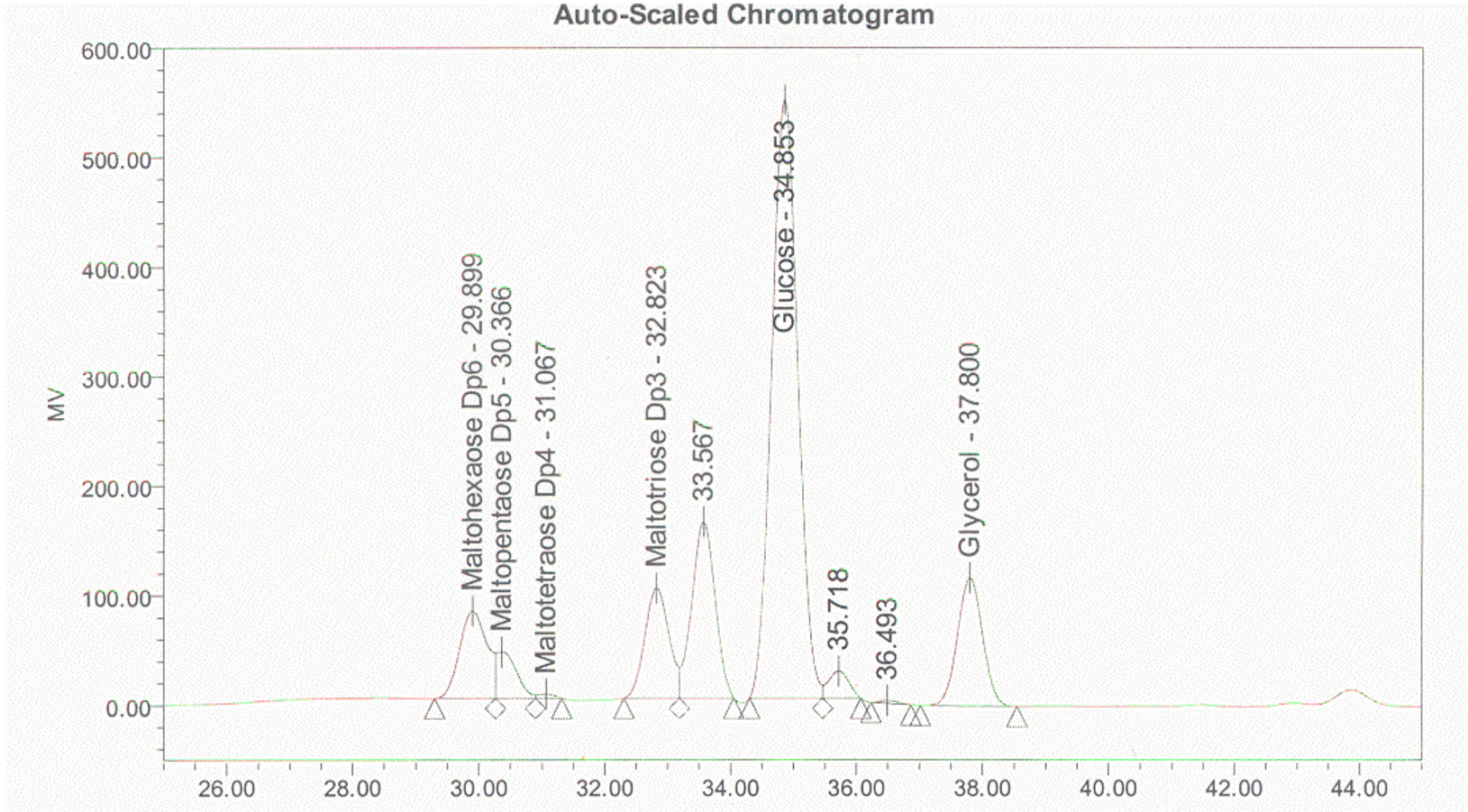
Dietary Fibre (18)

- LC chromatogram



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Dietary Fibre (19)

- **Results (AOAC 2001.03)**

%TDF = %(IDF + HMWRMD) + %LMWRMD

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Dietary Fibre (20)

AOAC official methods for functional fibre

Functional fibre	Commercial name	Test Method
Beta-glucan	Imprime PGG®	AOAC 995.16
Oligofructose	Raftilose®, OliggoFiber™	AOAC 997.08 or 999.03
Fructooligosaccharides	Neosugar, Actilght®	AOAC 997.08 or 999.03
Polydextrose	Litesse®	AOAC 2000.11
Galactooligosaccharides	Yacult, Borculo Whey Products	AOAC 2001.02
Glucooligosaccharides	BioEurope	AOAC 999.03 or 997.08
Resistant maltodextrin	Fibersol-2	AOAC 2001.03
Resistant starch	C*Actistar	AOAC 2002.02

Dietary Fibre (21)

- **Methods are applicable for specific functional fibre**
- **AOAC 2001.03 can provide good recoveries for different functional fibre, except glucooligosaccharides and resistant starch**

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Dietary fibre - Points to note

- Results are method dependent
- Functional fibre would increase the TDF results but may not be 100%
- AOAC 2001.03 may give higher TDF results but with higher testing cost
- Definition of “0”: ≤ 1 g/100g

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Dietary fibre – Available proficiency test

- FAPAS
- AOAC
- LGC

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Summary

- **Energy, Total fat, Protein, Carbohydrates**
- **Tests involved:**
  - ✓ **Total fat**
  - ✓ **Protein**
  - ✓ **Water**
  - ✓ **Ash**
  - ✓ **Dietary Fibre**
  - ✓ **Organic acids (optional)**
  - ✓ **Ethanol (optional)**

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Thank You

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