Nutrient Test Methods
(Part 1)

Seminar on Food Nutrition
Labelling Test Method
Outline

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

- Method Guidance Notes on Nutrition Labelling and Nutrition Claims, CFS
- 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
Core Nutrients (1 + 7)

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

- Dietary Fibre
- Cholesterol
- Unsaturated fatty acids
- Minerals
- Vitamins
- etc.
Analysis of Energy
**Energy**

By calculation

\[
\text{kcal [kJ]} \text{ per 100g of food} = 4 [17] \times \text{available carbohydrates} + 4 [17] \times \text{protein} + 9 [37] \times \text{total fat} + 7 [29] \times \text{ethanol} + 3 [13] \times \text{organic acids}
\]

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

Reference: Codex Guidelines on Nutrition Labelling
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
Energy - Available proficiency test

AOAC
Analysis of Total Fat
**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
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
Total Fat (3)

Analysis

Homogenous Sample → Drying, Acid / Alkaline hydrolysis → Solvent extraction, e.g. Soxhlet, mojonnier → Evaporate solvent → Total fat (weight of residue)
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 \((1 \times 0.5\%) = 0.005\) g of fat
**Total Fat (5)**

- Apparatus for blending samples
Total Fat (6)

- Freeze-dryer
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
Total Fat (8)

- Acid-hydrolysis
Total Fat (9)

- Extraction
Total Fat (10)

🌟 Determine the weight of residue

Drying to constant weight

Prolong heating may increase weight of fat, due to oxidation
Total Fat - Points to note

- Appropriate method is important
- Acid hydrolysis can produce higher results for cereal products
Total Fat - Available proficiency test

- FAPAS
- AOAC
- LGC
- AOCS
Analysis of Protein
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
Protein (2)

Analysis (TKN)

1. Homogenous Sample
2. Digestion
3. Steam distillation
4. Titration
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 \((1 \times 0.5\%) / 6.25 = 0.0008\) g of N
\((\sim 0.06\) mMole) \((0.6\) ml x 0.1M HCl)
Protein (4)

**Digestion**

Reagents: conc. H$_2$SO$_4$, Na$_2$SO$_4$ or K$_2$SO$_4$ and catalyst (e.g. CuSO$_4$·5H$_2$O, TiO$_2$)

H$_2$SO$_4$ : Na$_2$SO$_4$  \( \rightarrow \)  2:1 (initial)  \( \rightarrow \)  1:1 (final)

Temperature: ~420 °C

Time: ~2 hr

Appearance of final solution: clear solution
Protein (5)

- Digestion
Protein (6)

- **Distillation**
  - Add NaOH → strongly alkaline
  - Distill NH₃ into 1) standardized HCl or 2) boric acid

- **Titration**
  1) with NaOH
  2) with HCl
Protein (7)

Distillation & titration
Protein (8)

- Analysis by Combustion
  results similar to TKN

- Examples
  AOAC 992.15 for meat and meat product
  AOAC 992.23 for cereal grains
Protein (9)

Analysis (Combustion)

Homogenous Sample → Combustion instrument
i) Furnace, temp $\geq 950$ °C
ii) Isolation system
iii) Detection system → Read result, %N
Protein (10)

- **Sample preparation**
  representative and homogenous sample
  sample size $\geq 200$ mg

- **Instrument**
  Working range
  e.g. 0.5% to 60% protein $=>$ 0.08% to 10% N
Protein (11)

Combustion Instrument
Protein (12)

- Analysis by sum of amino acids after hydrolysis of proteins

- may involve huge amount of validation work
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
Protein - Available proficiency test

- FAPAS
- AOAC
- LGC
Analysis of Ethanol
Ethanol

- Gas Chromatographic method

- Examples
  AOAC 986.12 for canned salmon
  AOAC 984.14 for beer
Analysis of Organic Acids
Organic acid (1)

- Liquid Chromatographic method

- Examples
  AOAC 986.13 for cranberry juice cocktail and apple juice
  GB 5009.157 for foods
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)
Analysis of Carbohydrates
Carbohydrates (1)

Total Carbohydrates = Available carbohydrates + dietary fibre
Available Carbohydrates

Calculated by Difference:

$$100 - \text{(protein + fat + water + ash + ethanol + dietary fibre)} \%(\text{w/w})$$

Direct analysis
Carbohydrates - Points to note

- Includes sugar alcohols

  => use same conversion factor as carbohydrates for energy calculation
Carbohydrates - Available proficiency test

AOAC
Analysis of Water (moisture)
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.
Water (2)

- Analysis - Air oven method

- Examples
  - AOAC 935.29 for malt
  - AOAC 950.46 for meat
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
Water (4)

Analysis for air or vacuum oven method

- Homogenous Sample
- Drying in oven
  - i) Air oven or
  - ii) Vacuum oven
- Weighing samples
- Constant weight?
  - YES: Calculate the results
  - NO: Repeat the process
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 \((1 \times 0.1\%) = 0.001\) g (1 mg) of water
Water (6)

Sample preparation
Water (7)

Oven conditions

Air Oven method:
- **temp:** depends on the method, usually about 100 °C
- **pressure:** atmospheric pressure
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
Water (9)

- Drying time
  Depends on the temperature used
  ~ 4 – 18 hours

  too long heating time ==> the weight increase due to oxidation
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
Water (11)

Desiccator
Water (12)

Analysis – Co-Distillation Method
For containing significant amount of volatile substance other than water

Examples
AOAC 969.19 for cheeses
AOAC 986.21 for spices
Water -
Available proficiency test

- FAPAS
- AOAC
- LGC
Analysis of Ash
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
Ash (2)

Analysis

- Homogenous Sample
- Pre-drying ~ 100 - 200 °C
- Add a few drops of H₂O₂, water or nitric acid
- Ashing ~ 500 – 600 °C
- Constant weight or light grey ash?
  - NO
  - YES
- Calculate the results
- Weigh sample and observe ash colour
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
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
Ash (5)

Results

White or light grey ash => no carbon remains

Blank may be required for correction
Ash (6)

- Weighing and ashing
Ash (7)

- Thermogravimetric analyzer

Obtain moisture and ash results automatically
Ash (8)

Thermogravimetric analyzer
Ash -
Available proficiency test

- FAPAS
- AOAC
- LGC
Analysis of Dietary Fibre
HK Regulation - Any fibre analyzed by means of any official methods adopted by AOAC International
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)
**Dietary Fibre (3)**

*Analysis (AOAC 985.29)*

1. **Homogenous Sample in duplicate**
2. **Enzymatic Digestion**
   - i) α-amylase
   - ii) protease
   - iii) amyloglucosidase
3. **Precipitation and Filtration**
4. **Filtrate**
5. **Residue**
   - Total Dietary Fibre = Weight of Residue - Ash - Protein

AOAC 2001.03

Go to AOAC 2001.03
Dietary Fibre (4)

Analysis (AOAC 2001.03)

Filtrate → Concentration and Desalting by ion exchange column → Analysis by HPLC

Total Dietary Fibre = [Weight of Residue - Ash - Protein] + [weight of low MW soluble fibre determined by HPLC]
Dietary Fibre (5)

Empirical Methods

Results are valid only if the procedure is strictly followed.
## Dietary Fibre (6)

### Enzyme purity

<table>
<thead>
<tr>
<th>Test sample</th>
<th>Activity tested</th>
<th>Sample weight, g</th>
<th>Expected recovery, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Citrus pectin</td>
<td>Pectinase</td>
<td>0.1</td>
<td>95-100</td>
</tr>
<tr>
<td>Stractan (larch gum)</td>
<td>Hemicellulase</td>
<td>0.1</td>
<td>95-100</td>
</tr>
<tr>
<td>Wheat Starch</td>
<td>Amylase</td>
<td>1.0</td>
<td>0-1</td>
</tr>
<tr>
<td>Corn Starch</td>
<td>Amylase</td>
<td>1.0</td>
<td>0-2</td>
</tr>
<tr>
<td>Casein</td>
<td>Protease</td>
<td>0.3</td>
<td>0-2</td>
</tr>
<tr>
<td>β-Glucan (barley gum)</td>
<td>β-Glucanase</td>
<td>0.1</td>
<td>95-100</td>
</tr>
</tbody>
</table>
Sample preparation

homogenious 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
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
Dietary Fibre (9)

- Preparing for digestion
**Dietary Fibre (10)**

- **Enzymatic digestion**
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
Dietary Fibre (12)

- Precipitation
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
Dietary Fibre (14)

- Filtration
Dietary Fibre (15)

Residues collected
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)

\[ \text{TDF} = \text{weight of dried residue} - \text{ash} - \text{protein} \]
\[ (\text{IDF + HMWRMD}) \]

Blank value correction
Dietary Fibre (17)

Determination of low MW resistant maltodextrin (LMWRMD):
- concentrate the filtrate
- remove salt from buffer by ion-exchange column
- analyze by HPLC with RI detector => LMWRMD
Dietary Fibre (18)

- LC chromatogram
Dietary Fibre (19)

Results (AOAC 2001.03)

\[ %TDF = \% (IDF + HMWRMD) + \%LMWRMD \]
## AOAC official methods for functional fibre

<table>
<thead>
<tr>
<th>Functional fibre</th>
<th>Commercial name</th>
<th>Test Method</th>
</tr>
</thead>
<tbody>
<tr>
<td>Beta-glucan</td>
<td>Imprime PGG®</td>
<td>AOAC 995.16</td>
</tr>
<tr>
<td>Oligofructose</td>
<td>Raftilose®, OliggoFiber™</td>
<td>AOAC 997.08 or 999.03</td>
</tr>
<tr>
<td>Fructooligosaccharides</td>
<td>Neosugar, Actilight®</td>
<td>AOAC 997.08 or 999.03</td>
</tr>
<tr>
<td>Polydextrose</td>
<td>Litesse®</td>
<td>AOAC 2000.11</td>
</tr>
<tr>
<td>Galactooligosaccharides</td>
<td>Yacult, Borculo Whey Products</td>
<td>AOAC 2001.02</td>
</tr>
<tr>
<td>Glucoooligosaccharides</td>
<td>BioEurope</td>
<td>AOAC 999.03 or 997.08</td>
</tr>
<tr>
<td>Resistant maltodextrin</td>
<td>Fibersol-2</td>
<td>AOAC 2001.03</td>
</tr>
<tr>
<td>Resistant starch</td>
<td>C*Actistar</td>
<td>AOAC 2002.02</td>
</tr>
</tbody>
</table>
Dietary Fibre (21)

- Methods are applicable for specific functional fibre.
- AOAC 2001.03 can provide good recoveries for different functional fibre, except glucoooligosaccharides and resistant starch.
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”: \( \leq 1 \text{ g/100g} \)
Dietary fibre – Available proficiency test

- FAPAS
- AOAC
- LGC
Summary

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