Analysis of Dietary Fibre
HK Regulation

- Dietary fibre means any fibre analyzed by means of any official methods adopted by AOAC International.
## AOAC Official Methods

<table>
<thead>
<tr>
<th>Method</th>
<th>Description</th>
<th>Method</th>
</tr>
</thead>
<tbody>
<tr>
<td>AOAC 985.29</td>
<td>Total Dietary Fibre in Foods</td>
<td>Enzymatic-Gravimetric Method</td>
</tr>
<tr>
<td>AOAC 993.19</td>
<td>Soluble Dietary Fibre in Food and Food Products</td>
<td>Enzymatic-Gravimetric Method</td>
</tr>
<tr>
<td>AOAC 991.42</td>
<td>Insoluble Dietary Fibre in Foods and Food Products</td>
<td>Enzymatic-Gravimetric Method</td>
</tr>
<tr>
<td>AOAC 991.43</td>
<td>Total, Soluble, and Insoluble Dietary Fibre in Foods</td>
<td>Enzymatic-Gravimetric Method</td>
</tr>
<tr>
<td>AOAC 992.16</td>
<td>Total Dietary Fibre (Applicable to determination of total fibre in</td>
<td>Enzymatic-Gravimetric Method</td>
</tr>
<tr>
<td>etc</td>
<td>cereals, beans, vegetables and fruits)</td>
<td></td>
</tr>
</tbody>
</table>
AOAC 985.29

Duplicate test portions of sample

Enzymatic digestion
i) α-amylase
ii) protease
iii) amyloglucosidase

Precipitation and filtration (residue)

One residue for protein analysis
One residue for ash analysis

Total dietary fibre = weight (residue) – weight (ash + protein)
**AOAC 985.29**

- Homogenize and dry sample (freeze-dry is recommended).

- Defat with petroleum ether if >10% fat content, otherwise false high results.

- Weigh duplicate test portions (difference in weight should not >20 mg).
AOAC 985.29

Preparing for digestion
AOAC 985.29
Preparing for digestion
AOAC 985.29

α-Amylase: gelatinize

- Add phosphate buffer (pH 6.0, 50 mL) to sample.

- Adjust to pH 6.0 ± 0.2.

- Add enzyme, incubate at 95 – 100 °C.

- 30 minutes in water bath.
AOAC 985.29

Protease: remove protein

- Cool to room temperature.
- Adjust to pH 7.5 ± 0.2.
- Add enzyme, incubate at 60 °C for 30 minutes.
AOAC 985.29

Amyloglucosidase: remove starch

- Cool.
- Adjust to pH 4.0 – 4.6.
- Add enzyme, incubate at 60 °C for 30 minutes.

*Note: final solution volume is about 70 mL.*
AOAC 985.29

Summary

- pH 6.0 ± 0.2, α-amylase, 95 – 100 °C, 15 - 30 min.

- pH 7.5 ± 0.2, protease, 60 °C, 30 min.

- pH 4.0 – 4.6, amyloglucosidase, 60 °C, 30 min.
AOAC 985.29

- Add 280 mL 95 % ethyl alcohol (60 °C).
- Let precipitate form at room temperature for 60 minutes.
- Collect the residues (soluble fibre + insoluble fibre) in pre-weight crucibles.
AOAC 985.29

Precipitation
AOAC 985.29

Filtration
**AOAC 985.29**

- One test portion for protein, using N x 6.25 as conversion factor.

- Incinerate second test portion at 525 °C for 5 hours.

  \[
  \text{Total dietary fibre} = \frac{[\text{weight residue} - \text{protein} - \text{ash} - \text{blank}]}{\text{weight test portion}}
  \]

  Weight residue = average of duplicate
  Weight test portion = average of duplicate
**AOAC 985.29**

Test for enzyme purity every half yearly

<table>
<thead>
<tr>
<th>Test sample</th>
<th>Activity tested</th>
<th>Test portion (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>
# AOAC Method 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>Resistant maltodextrin</td>
<td>Fibersol-2</td>
<td><strong>AOAC 2001.03</strong></td>
</tr>
<tr>
<td>Resistant starch</td>
<td>C*Actistar</td>
<td>AOAC 2002.02</td>
</tr>
</tbody>
</table>
AOAC 2001.03

Dietary Fiber Containing Supplemented Resistant Maltodextrin (RMD)

High MW RMD by Method 985.29 and Low MW RMD by HPLC
AOAC 2001.03

Filtrate (by 985.29) → i) Concentrate → ii) Desalt → Analysed by LC

Total dietary fibre = [weight of residue - protein – ash - blank] + [weight of low MW soluble fibre determined by HPLC]
AOAC 2001.03

- Only a portion of the resistant maltodextrin is precipitated in the aqueous ethanol when Method 985.29 is applied to foods containing resistant maltodextrin.
In *Method 2001.03*, resistant maltodextrin that are soluble in aqueous ethanol are desalted, concentrated and measured by liquid chromatography.
Desalting

- Ion exchange column (OH-type and H-type)
AOAC 2001.03

Total dietary fibre =

fibre as measured by Method 985.29 plus
low molecular weight resistant maltodextrin

Note: low molecular weight resistant maltodextrin refers to resistant maltodextrin that are soluble in the aqueous ethanol in Method 985.29.
Calculation of available carbohydrate when method 2001.03 is used

- Available carbohydrate = 100 – [moisture + ash + protein + fat + alcohol + (soluble dietary fibre + insoluble dietary fibre) + low molecular weight resistant maltodextrin]

Note: low molecular weight resistant maltodextrin refers to resistant maltodextrin that is measured by AOAC 2001.03.
Recoveries for other functional fibres when tested by AOAC 2001.03

<table>
<thead>
<tr>
<th>Functional fibre</th>
<th>Recovery (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>β-Glucan</td>
<td>101*</td>
</tr>
<tr>
<td>Fructooligosaccharides</td>
<td>98</td>
</tr>
<tr>
<td>Polydextrose</td>
<td>117</td>
</tr>
<tr>
<td>Galactooligosaccharides</td>
<td>111</td>
</tr>
<tr>
<td>Glucooligosaccharides</td>
<td>46</td>
</tr>
<tr>
<td>Resistant maltodextrin</td>
<td>88</td>
</tr>
</tbody>
</table>

* Recovery of insoluble dietary fibre and soluble dietary fibre is particular high.
Points to note

- Definition of “0”: ≤1 g/100 g.
- Results are method dependent.
- Functional fibre should be included in the calculation of available carbohydrate.
- AOAC 2001.03 may give higher TDF results but with higher testing cost.
Available proficiency test

- FAPAS
- AOAC
- LGC
Thank You