SNAPSHOOT
ENGINEERED FOR A SAFER TOMORROW

현장을 이해하고, 필요한 솔루션을 연결합니다.

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제품의 규격과 성능, 인증 및 적용 조건을 확인하기 위한 자료입니다.

ABOUT SNAPSHOOT

제품 공급을 넘어, 현장에 맞는 해결책으로

스냅슛은 산업현장의 작업 환경과 요구 조건을 바탕으로
적합한 제품과 시스템을 제안합니다. 현장 실사를 통해 설치 조건을 확인하고,
공급부터 시공·설치 및 사용 교육까지 필요한 과정을 함께합니다.

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Megazyme

www.megazyme.com

AVAILABLE CARBOHYDRATES and DIETARY FIBER

ASSAY PROCEDURE

K-ACHDF 06/18

(100 Assays of each per Kit)



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INTRODUCTION:

A need for information on the carbohydrate composition of foods for diabetics prompted McCance and Lawrence[1] to attempt to measure this to gain results that would be of biological significance. They divided the carbohydrates in foods into two broad groups, 'available' and 'unavailable'. The available carbohydrates, that is sugar plus starch, were defined as those that are digested and absorbed by the human small intestine and which are glucogenic. The unavailable carbohydrates were defined as those that are not digested by the endogenous secretions of the human digestive tract. These are now generally referred to as dietary fiber.

The concept of 'available' and 'unavailable' carbohydrates drew attention to the fact that some carbohydrates are not digested and absorbed in the small intestine but rather reach the large bowel where they are fermented. In the FAO/WHO report on "Carbohydrates in Human Nutrition",[2] it was stated that these terms can be misleading since the so-called 'unavailable' carbohydrate can, in fact, provide energy to the body through fermentation. This committee recommended use of the term 'glycemic' (i.e. providing carbohydrate for metabolism) to replace 'available'. However, this term has not gained widespread acceptance, possibly because there is some confusion with the well recognised term 'glycemic index'. For this reason, we have decided to continue using the term 'available carbohydrates' for carbohydrates that are digested and absorbed by the human small intestine; these include D-glucose, D-fructose, sucrose, maltodextrins, non-resistant starch and the D-glucose component of lactose; all measured as D-glucose plus D-fructose following enzymic hydrolysis.

Dietary fiber is a mixture of complex organic substances including a range of hydrophilic compounds such as soluble and insoluble polysaccharides and non-digestable oligosaccharides as well as non-swellable, more or less hydrophobic compounds such as cutins, suberins and lignins. The procedures for the determination of total dietary fiber as outlined in this booklet are based on the methods of Lee *et al.*[3] and Prosky *et al.*[4] (AOAC Official Method 991.43, AACC Method 32-07.01). These methods do not measure non-digestible oligosaccharides, as these remain soluble in the ethanolic solution used to precipitate high molecular weight dietary fiber components.

PRINCIPLE:

Dietary fiber (DF) is determined on duplicate samples of dried and defatted [if fat content is > 10% (w/w)] material. Samples are incubated at 80°C with heat stable α-amylase to give gelatinisation, hydrolysis and depolymerisation of non-resistant starch; incubated at 60°C with protease (to solubilise and depolymerise proteins); and with amyloglucosidase (to hydrolyse starch fragments to D-glucose). This solution is allowed to cool to approx. 40°C **and a sample (1.0 mL) is removed for available carbohydrate determination**. The

remainder of the solution is treated with four volumes of ethanol to precipitate soluble fiber and remove depolymerised protein and D-glucose (from starch). The residue is filtered; washed with 78% (v/v) ethanol, 95% (v/v) ethanol, and acetone; dried and weighed. One duplicate residue is analysed for protein and the other is incubated at 525°C to determine ash. DF is the weight of the filtered and dried residue less the weight of the protein and ash.

In the current format for dietary fiber, incubation with α-amylase is performed at 80°C instead of 100°C, as used in AOAC TDF Method 991.43. Results obtained for most samples are very similar to those obtained with AOAC Method 991.43 (see Table 1, page 16). However, there is less hydrolysis of resistant starch[5] (particularly RS_2 and RS_3),[6] and thus the determined RS values are somewhat closer to those obtained with AOAC Official Method 2002.02 ('Resistant starch in starch and plant materials').[7] Another modification involves the use of acetic acid in place of hydrochloric acid to effect the pH change before the addition of amyloglucosidase. This has the advantage that the pK_a of acetic acid is near the final pH required for the amyloglucosidase incubation step. Addition of the stated amount of acetic acid **always** gives a reaction solution with a final pH of 4.1-4.5. Also, the incubations are performed in a sealed Duran® bottle. This ensures that there is no loss of liquid volume during the incubations and that insoluble residues do not stick to the side of the incubation container, making recovery simpler and more reproducible.

The sample of solution removed for available carbohydrate determination is centrifuged, an aliquot is diluted with sodium maleate buffer (pH 6.0), and a sub-aliquot is incubated with a mixture of sucrase/maltase plus β-galactosidase to hydrolyse sucrose and lower degree-of-polymerisation (DP) maltosaccharides (if present in the sample) to D-fructose and D-glucose and lactose to D-glucose and D-galactose.[6] This mixture is analysed for D-glucose and D-fructose using hexokinase plus glucose-6-phosphate dehydrogenase, followed by phosphoglucose isomerase.

The Megazyme Available Carbohydrates and Dietary Fiber assay kit (**K-ACHDF**) contains high purity enzymes devoid of interfering activities, and the activities of the enzymes are standardised. The importance of standardised α-amylase activity in the measurement of total dietary fiber is well recognised. Amyloglucosidase supplied by Megazyme is essentially devoid of cellulase (which if present can lead to depolymerisation and underestimation of β-glucan). All enzymes supplied in the Megazyme Available Carbohydrates and Dietary Fiber assay kit are in a ready-to-use, stabilised, liquid form.

SCOPE:

Applicable to cereal grains, fruit and vegetables, cereal and fruit products and foods. Particularly useful for the analysis of samples containing resistant starch (specifically RS_2 and RS_3).

ENZYME PURITY AND STANDARDISATION:

The effectiveness and purity of Megazyme α-amylase, protease and amyloglucosidase have been evaluated using the standards recommended in AOAC Method 985.29 and 991.43, and AACC Method 32-05.01. Thermostable α-amylase from Megazyme (cat. no. **E-BLAAM**) has an activity of 3,000 U/mL (Ceralpha method); protease is supplied at a concentration of 50 mg/mL (~ 350 tyrosine U/mL); and amyloglucosidase is supplied at a concentration of 200 U/mL (*p*-nitrophenyl β-maltoside substrate) (or ~ 3,300 U/mL on soluble starch). This amyloglucosidase activity is 150% the concentration traditionally used in TDF assays, so that 0.2 mL (instead of 0.3 mL) is used in the assay. Megazyme amyloglucosidase (cat. no. **E-AMGDF**) is essentially devoid of cellulase.

SAFETY:

The general safety measures that apply to all chemical substances should be adhered to.

For more information regarding the safe usage and handling of this product please refer to the associated SDS that is available from the Megazyme website.

KITS:

Kits suitable for performing 100 assays of each are available from Megazyme. The kits contain the full assay method plus:

Bottle 1: Buffer (11 mL, pH 7.6) plus sodium azide (0.02% w/v) as a preservative.
Stable for > 3 years at 4°C.

Bottle 2: $NADP^+$ plus ATP.
Stable for > 5 years below -10°C.

Bottle 3: Thermostable α-amylase (6 mL, 3,000 U/mL) (Megazyme cat. no. **E-BLAAM**).
Stable for > 3 years at 4°C.

Bottle 4: Protease (10 mL, ~ 350 U/mL) (Megazyme cat. no. **E-BSPRT**).
Stable for > 3 years below -10°C.

Bottle 5: Amyloglucosidase (20 mL, ~ 3,300 U/mL) (Megazyme cat. no. **E-AMGDF**).
Stable for > 3 years at 4°C.

Bottle 6: Sucrase plus β-galactosidase, freeze-dried powder.
Stable for > 3 years below -10°C.

Bottle 7: Hexokinase plus glucose-6-phosphate dehydrogenase suspension, 2.25 mL.
Stable for > 3 years at 4°C.

Bottle 8: Phosphoglucose isomerase suspension (2.25 mL).
Stable for > 3 years at 4°C.

Bottle 9: D-Glucose plus D-fructose standard solution (5 mL, 0.2 mg/mL of each sugar).
Stable for > 3 years at 4°C.

PREPARATION OF REAGENT SOLUTIONS/SUSPENSIONS:

1. Use the contents of bottle 1 as supplied.
Stable for > 3 years at 4°C.

2. Dissolve the contents of bottle 2 in 12 mL of distilled water. **Stable for > 1 year at 4°C** or stable for > 2 years below -10°C (to avoid repetitive freeze/thaw cycles, divide into appropriately sized aliquots and store in polypropylene tubes).

3, 4 & 5. Use the contents of bottles 3, 4 and 5 as supplied.
Stable for > 3 years at 4°C or below -10°C (bottle 4).

6. Dissolve the contents of bottle 6 in 10.5 mL of distilled water. Divide into aliquots of approx. 5 mL. Store below -10°C in polypropylene tubes between use and keep cool during use if possible.
Stable for > 2 years below -10°C.

7 & 8. Use the contents of bottles 7 and 8 as supplied. Before opening for the first time, shake the bottles to remove any enzyme that may have settled on the rubber stopper. Subsequently, store the bottles in an upright position. **Swirl the bottle to mix contents before use.**
Stable for > 3 years at 4°C.

9. Use the contents of bottle 9 as supplied.
Stable for > 3 years at 4°C.

REAGENTS NOT SUPPLIED:

1. Ethanol, 95% (v/v).
2. Ethanol, 78% (v/v). Place 821 mL 95% v/v ethanol (or IMS) into a 1 L volumetric flask. Dilute to volume with deionised water. Mix well. Check the level and if necessary add more deionised water to bring it back up to the 1 L mark.
3. Acetone, reagent grade.
4. Deionised water.

5. ***Celite***®, analytical grade, in 100 g or 500 g packages, is available separately (cat. no. **G-CEL-100G** or cat. no. **G-CEL-500G**).
6. Cleaning solution, Micro (International Products Corp., Trenton, NJ). Make a 2% (v/v) solution with deionised water.
7. MES/TRIS buffer, 0.05 M each, pH 8.0 at 23°C. Dissolve 19.52 g 2(*N*-morpholino) ethanesulfonic acid (MES) (cat. no. **B-MES250**) and 14.2 g tris(hydroxymethyl)aminomethane (TRIS) (cat. no. **B-TRIS500**) in 1.7 L deionised water.

   Adjust pH to 8.0 with 6.0 M NaOH. Dilute to 2 L with water. **NOTE:** the pH of buffer must be adjusted at approx. 23°C. Stable for ~ 1 month at 4°C.
8. Sodium maleate buffer, 0.1 M, pH 6.2. Dissolve 11.6 g of maleic acid (Sigma cat. no. M0375) in 900 mL of distilled water and adjust the pH to 6.2 with 2 M NaOH (approx 80 mL is required). Add sodium azide (0.2 g) and adjust the volume to 1 L. Stable for > 1 year at room temperature.

A. DIETARY FIBER DETERMINATION

a. EQUIPMENT:

1. Duran® bottles (250 mL volume).
2. Fritted crucible, Corning® No. 36060 Büchner, fritted disk, Pyrex® 60 mL, pore size, coarse, ASTM 40-60 µm, or equivalent. Prepare as follows:
   a. Ash overnight at 525°C in muffle furnace.
   b. Remove Celite and ash material by using a vacuum.
   c. Soak in 2% (v/v) Micro cleaning solution (reagent 6) at room temperature for 1 h.
   d. Rinse crucibles with water and then deionised water.
   e. For final rinse, use 15 mL acetone and air dry.
   f. Add approx. 1.0 g Celite to dried crucibles and dry at 130°C to constant weight.
   g. Cool crucible in desiccator for approx. 1 h and record weight of crucible containing Celite.
3. Filtering flask, heavy-walled, with 1 L side arm.
4. Rubber ring adaptors for use on filtering flasks.
5. Vacuum source: vacuum pump or aspirator with regulator capable of regulating vacuum.
6. Water bath, shaking, large-capacity (20-24 L) with covers; capable of maintaining temperature of 100°C; equipped with automatic timers for on-off operation.
7. Balance, 0.1 mg accuracy.

8. Ovens, two, mechanical convection, set at 103 ± 2°C and 130 ± 3°C.
9. Desiccator, airtight, with SiO_2 or equivalent desiccant. Desiccant dried biweekly overnight in 130°C oven.
10. pH meter.
11. Pipettors and tips, 50-200 µL and 5 mL capacity.
12. Dispensers
    a. 15 ± 0.5 mL for 78% (v/v) EtOH, 95% ethanol, and acetone.
    b. 40 ± 0.5 mL for buffer.
13. Measuring cylinder, 500 mL.
14. Magnetic stirrers and stirring bars.
15. Rubber spatulas.
16. Muffle furnace, 525 ± 5°C.
17. Microfuge, capable of 12,000 *g*.

b. PROCEDURE (DIETARY FIBER):

1. Blanks

With each assay, run two blanks along with samples to measure any contribution from reagents to residue.

2. Samples

a. Weigh duplicate 1.000 ± 0.005 g samples accurately into 250 mL Duran® bottles.
b. Add 40 mL MES-TRIS buffer solution (pH 8.0; Reagents not supplied, '7', page 5) to each bottle. Add magnetic stirring bar to each bottle. Stir on magnetic stirrer until sample is completely dispersed in solution (this prevents lump formation, which would make sample inaccessible to enzymes).

3. Incubation with heat-stable α-amylase

a. Add 50 µL heat-stable α-amylase solution, while stirring at low speed.
b. Seal each bottle with plastic caps.
c. Place sealed bottles in shaking water bath at 80°C, and incubate for 35 min with continuous agitation. Start timing once all bottles are in hot water bath.

4. Cool

a. Remove all of the bottles from the water bath.
b. Adjust the temperature of water bath to 60°C by draining some of the hot water from water bath and adding cold water.

5. Incubation with protease

a. Allow the samples in the sealed bottles to cool to about 60°C and then add 100 µL of protease solution to each bottle

one at a time (removing caps from the bottles one at a time and replacing these as soon as the enzyme has been added to minimise evaporative loss).

b. Incubate bottles in a shaking water bath at 60 ± 1°C, with continuous agitation for 30 min. Start timing when temperature of water bath reaches 60°C.

6. pH adjustment

a. Remove the sample bottles one at a time from the water bath.

b. Remove the cap from the first bottle and immediately add 5 mL of 3 M acetic acid solution into the bottle while stirring the solution on a magnetic stirrer (the pH will be 4.1-4.5). No pH adjustment will be necessary.

7. Incubation with amyloglucosidase

a. Immediately add 200 µL of amyloglucosidase solution to the bottle with continuous stirring of the contents on a magnetic stirrer. Immediately cap the bottle.

b. Perform these same operations on each of the other bottles one at a time.

c. Incubate the bottles in a shaking water bath at 60°C for approx. 30 min, with constant agitation.

8. Removal of sample for ACH determination

a. Cool the contents of each bottle to approx 40°C by allowing them to stand at room temperature for approx. 10 min.

b. Shake the bottle contents to ensure that any liquid that has condensed on the neck of the bottle is mixed with the bulk solution.

c. Store the bottles at room temperature for a further 10 min to allow insoluble material to settle. Accurately remove a 1 mL sample from each bottle and transfer this to a 50 mL volumetric flask and adjust to volume with 100 mM sodium maleate buffer (pH 6.2; Reagents Not Supplied '8', page 5) (overall dilution of 50-fold) and mix well.

d. If the solution is very turbid, filter an aliquot (approx. 10 mL) through a Whatman® No. 1 (9 cm) filter paper (in most cases, this step is unnecessary).

e. Analyse 0.2 mL of this solution immediately **according to the procedure on page 13**, or store below -10°C.

9. Precipitation of soluble dietary fiber

Add 160 mL of 95% (v/v) EtOH pre-heated to 60°C to each of the bottles detailed in point 8. Mix well on a magnetic stirrer and then remove the magnetic stirrer bar. Let the precipitate form at room temperature for 60 min.

10. Filtration setup

a. Tare crucible containing Celite to nearest 0.1 mg.

b. Wet and redistribute bed of Celite in crucible using approx. 3 mL of distilled water.

c. Apply suction to crucible to draw Celite onto fritted glass as an even mat.

11. **Filter sample mixture** from Step 9 through crucible into a filtration flask.

12. Wash the residue successively with three 15 mL aliquots of 78% (v/v) EtOH, two 15 mL aliquots of 95% (v/v) EtOH, and two 15 mL aliquots of acetone. In some cases, gums may form during filtration, trapping liquid in the residue. If so, break the surface film with a spatula to improve filtration. Long filtration times can be avoided by careful intermittent suction throughout filtration.

13. Dry the crucible containing the residue overnight at 70°C in a vacuum oven, or at 105°C in an air oven.

14. Cool the crucibles in a desiccator and weigh them to the nearest 0.1 mg. Subtract the crucible plus Celite weight to determine the weight of the residue.

15. **Protein and ash determination**
    One residue from each type of fiber is analysed for protein, and the second residue of the duplicate is analysed for ash.
    a. Perform protein analysis on residue using Kjeldahl method. Use N x 6.25 as the conversion factor for all samples to calculate g of protein.
    b. For ash analysis, incinerate the second residue for 5 h at 525°C. Cool in desiccator and weigh to nearest 0.1 mg. Subtract crucible and Celite weight to determine ash. (See Note 3, page 9).

c. CALCULATIONS (DIETARY FIBER):

$$\text{Dietary Fiber (\% w/w)} = \frac{\frac{R_1 + R_2}{2} - p - A - B}{\frac{m_1 + m_2}{2}} \times 100$$

where:

R_1 = residue weight 1 from m_1

R_2 = residue weight 2 from m_2

m_1 = sample weight 1

m_2 = sample weight 2

p = protein weight from R_1

A = ash weight from R_2 and

B = blank

$$= \frac{BR_1 + BR_2}{2} - BP - BA$$

where:

BR = blank residue

BP = blank protein from BR_1

BA = blank ash from BR_2

NOTE: The calculations above can be simplified by using the Megazyme ***Mega-Calc***™, downloadable from where the product appears on the Megazyme website (www.megazyme.com).

d. NOTES:

1. Differences between this method and AACC methods 32-05 and 32-21 are as follows:
   a. Forty (40) mL MES-TRIS buffer, 0.05 M each, pH 8.0 at 23°C is used instead of 50 mL phosphate buffer, 0.08 M, pH 6.0. The pH of MES-TRIS buffer changes with temperature. A MES-TRIS buffer of pH 8.0 at 23°C changes to pH 7.4-7.6 at 60°C and pH 6.8 at 80°C. **Note** that the pH optimum of heat-stable α-amylase moves from pH 6.0 at 60°C toward pH 7.0 at 80°C.
   b. Incubations are performed in 250 mL sealed Duran® bottles at 80°C. This prevents evaporative loss of solution and also prevents drying of solid sample on the edge of the bottle. This, in turn, simplifies the removal of sample from the container (i.e. it does not have to be scraped from the glass surface).
   c. The volume of thermostable α-amylase used has been reduced from 200 µL to 50 µL due to the higher activity of the enzyme employed here.
   d. No pH adjustment is needed for protease action, thus no NaOH is added to the incubation mixture.
   e. Before adding amyloglucosidase, 5 mL of 3 M acetic acid is added to adjust the pH to 4.1-4.5. Unlike pH adjustment with 0.561 M HCl, acetic acid always gives the required pH for the amyloglucosidase incubation step; thus there is no need for pH checking of each sample.
   f. For TDF determination, the amount of 95% (v/v) EtOH added for the precipitation step is 160 mL instead of 280 mL. For SDF/IDF determination, the weight of filtrate and washing solution is adjusted to 60 g instead of 100 g. Thus, 240 mL of 95% (v/v) EtOH at 60°C is added. Alternatively, weigh the combined solution of filtrate and washing solution and add 4 vol. 95% EtOH. The total filtration volume is reduced to 300-320 mL with this modification (see Figures 1 and 2).
2. There is some indication that delay in washing IDF residues with 95% EtOH and acetone may cause inflated IDF values. Thus, it is recommended that the IDF residues are washed immediately after all free buffer solution is sucked through the filter.
3. In some samples a gum is formed, trapping liquid. If this occurs, break the layer of film with a spatula.

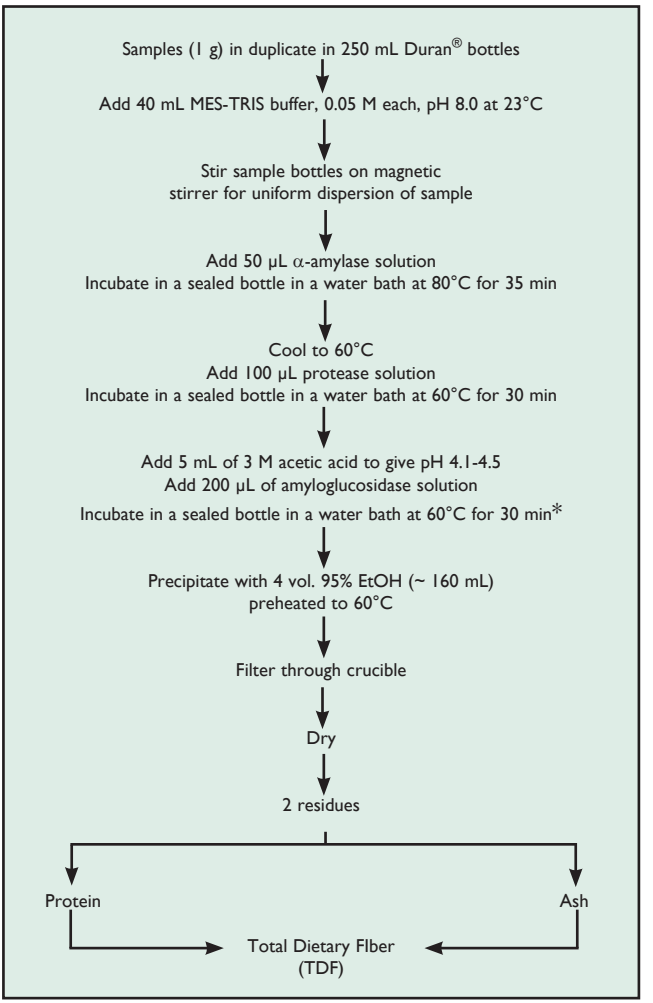


Figure 1. Analytical scheme for the total dietary fiber determination procedure.

* If a 1.0 mL aliquot is to be removed for available carbohydrate determination, then cool the bottle to approx. 40°C, shake to mix the contents, and allow the insolubles to settle for approx. 10 min. Proceed to the next step in the TDF assay with the remaining solution.

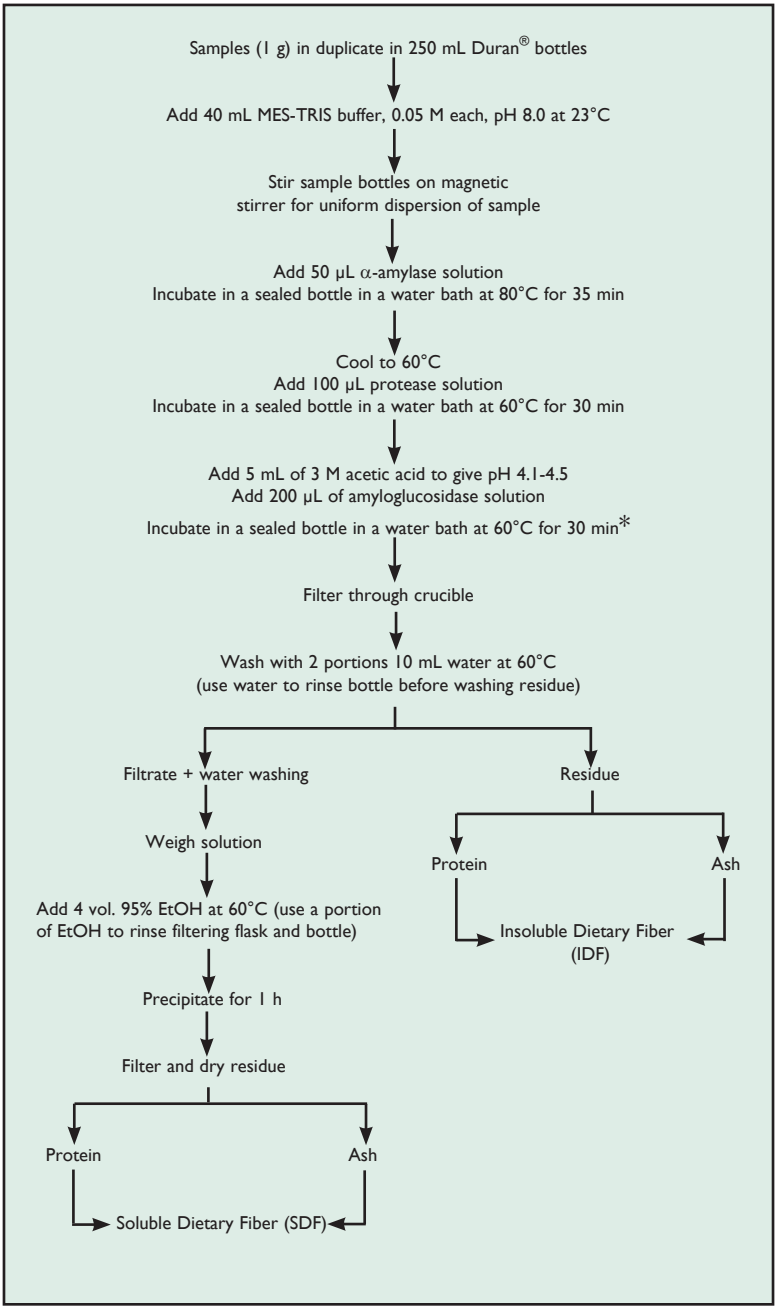


Figure 2. Analytical scheme for soluble and insoluble dietary fiber determination procedures.

* See comment under Figure 1.

e. REFERENCES:

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B. AVAILABLE CARBOHYDRATE DETERMINATION

a. EQUIPMENT (AVAILABLE CARBOHYDRATES):

1. Volumetric flasks (50 mL, 100 mL and 500 mL).
2. Disposable plastic cuvettes (1 cm light path, 3.0 mL).
3. Micro-pipettors, e.g. Gilson Pipetman® (20, 100 and 1000 µL).
4. Positive displacement pipettor, e.g. Eppendorf Multipette®
   - with 5.0 mL Combitip® (to dispense 0.1 mL aliquots of Buffer 1 and $NADP^+$/ATP solution).
   - with 25 mL Combitip® (to dispense 2.0 mL aliquots of distilled water).
5. Analytical balance.
6. Spectrophotometer set at 340 nm.
7. Vortex mixer (e.g. IKA® Yellowline Test Tube Shaker TTS2).
8. Stop clock.
9. Whatman® No. 1 (9 cm) filter papers.

b. PROCEDURE (AVAILABLE CARBOHYDRATES):

Wavelength: 340 nm
Cuvette: 1 cm light path (glass or plastic)
Temperature: ~ 25°C
Final volume: 2.52 mL (D-glucose)
2.54 mL (D-fructose)
Sample solution: 4-80 µg of D-glucose plus D-fructose per cuvette (in 0.10-2.00 mL sample volume)

Read against air (without a cuvette in the light path) or against water

Pipette into cuvettes	Blank	Sample
sample in maleate buffer (pH ~ 6.2) solution 6 (sucrase + β-galactosidase)	- -	0.20 mL 0.10 mL
Ensure that all of the solutions are delivered to the bottom of the cuvette. Mix the contents by gentle swirling, cap the cuvettes and incubate them at ~ 25°C for 60 min. **Add:**		
distilled water (at ~ 25°C) solution 1 (buffer) solution 2 ($NADP^+$/ATP)	2.30 mL 0.10 mL 0.10 mL	2.00 mL 0.10 mL 0.10 mL
Mix* and read the absorbances of the solutions (A_1) after approx. 3 min and start the reactions by addition of:		
suspension 7 (HK/G6P-DH)	0.02 mL	0.02 mL
Mix* and read the absorbances of the solutions (A_2) at the end of the reaction (approx. 5 min). If the reaction has not stopped after 5 min, continue to read the absorbances at 2 min intervals until the absorbances remain the same over 2 min**. **Then add:**		
suspension 8 (PGI)	0.02 mL	0.02 mL
Mix* and read the absorbances of the solutions (A_3) at the end of the reaction (approx. 8-10 min).		

* for example with a plastic spatula or by gentle inversion after sealing the cuvette with a cuvette cap or Parafilm®.

** if the absorbance continues to increase, this may be due to effects of colour compounds or enzymes in the sample. These interfering substances may be removed during sample preparation.

c. CALCULATION:

Determine the absorbance difference (A_2-A_1) for both blank and sample. Subtract the absorbance difference of the blank from the absorbance difference of the sample, thereby obtaining $\Delta A_{D\text{-glucose}}$.

Determine the absorbance difference (A_3-A_2) for both blank and sample. Subtract the absorbance difference of the blank from the absorbance difference of the sample, thereby obtaining $\Delta A_{D\text{-fructose}}$.

The values of $\Delta A_{D\text{-glucose}}$ and $\Delta A_{D\text{-fructose}}$ should as a rule be at least 0.100 absorbance units to achieve sufficiently accurate results.

The concentration of D-glucose and D-fructose can be calculated as follows:

$$c = \frac{V \times MW}{\varepsilon \times d \times v} \times \Delta A \times F \quad [g/L]$$

where:

V = final volume [mL]
MW = molecular weight of D-glucose or D-fructose [g/mol]
ε = extinction coefficient of NADPH at 340 nm
= 6300 [l x mol^{-1} x cm^{-1}]
d = light path [cm]
v = sample volume [mL]
F = dilution factor

It follows for D-glucose:

$$c = \frac{2.52 \times 180.16}{6300 \times 1.0 \times 0.2} \times \Delta A_{D\text{-glucose}} \times 50 \quad [g/L]$$

$$= 18.016 \times \Delta A_{D\text{-glucose}} \quad [g/L]$$

for D-fructose:

$$c = \frac{2.54 \times 180.16}{6300 \times 1.0 \times 0.2} \times \Delta A_{D\text{-fructose}} \times 50 \quad [g/L]$$

$$= 18.159 \times \Delta A_{D\text{-fructose}} \quad [g/L]$$

Available Carbohydrates (ACH) = D-Glucose + D-Fructose [g/L]

If the sample dilution is other than 50-fold (as used for solid samples; see page 7, point 8), the factor should be altered accordingly.

When analysing solid and semi-solid samples which are weighed out for sample preparation, the content (g/100 g) is calculated from the amount weighed as follows:

Content of D-glucose

$$= \frac{c_{D\text{-glucose}}\ [g/L\ \text{sample solution}]}{\text{weight}_{\text{sample}}\ [g/L\ \text{sample solution}]} \times 100 \quad [g/100\ g]$$

Content of D-fructose

$$= \frac{c_{D\text{-fructose}}\ [g/L\ \text{sample solution}]}{\text{weight}_{\text{sample}}\ [g/L\ \text{sample solution}]} \times 100 \quad [g/100\ g]$$

In the standard assay in which 1.00 g of sample is analysed, the final extraction volume is 45.9 mL (it was found empirically that in aqueous solution, 1 g of starch, D-glucose or sucrose occupies a volume of ~ 0.55 mL). If liquid samples are analysed, this extraction volume will change to 45.35 **plus** the volume of the sample analysed.

NOTE: These calculations can be simplified by using the Megazyme ***Mega-Calc***™, downloadable from where the product appears on the Megazyme website (www.megazyme.com).

d. SAMPLE PREPARATION EXAMPLES:

(i) Determination of available carbohydrates in grains, breakfast cereals and solid foodstuffs.

Mill plant materials to pass a 0.5 mm screen. Homogenise solid foodstuffs such as bread, pastries, chocolate, confectionery or candy in a mixer, meat grinder or mortar. Accurately weigh 1.00 g into a 250 mL Duran® bottle. Analyse as per the procedure detailed on page 6. If dry weight values are required, determine the moisture content on a representative sample of the material.

(ii) Determination of available carbohydrates in fruit and vegetables.

Dry approx. 200 g of chopped fruit or vegetable in a freeze-drier and mill to pass a 1.0 mm screen. Accurately weigh 1.00 g of dry material, or 4.00 g of homogenised fresh material (final extraction volume of 49.35 mL (45.35 + 4.00 mL), into the bottom of a 250 mL Duran® bottle. Analyse as per the procedure detailed on page 6.

(iii) Determination of available carbohydrates in preserves and jams.

Homogenise approx. 50 g of jam in a Waring® blender. Accurately weigh approx. 2.00 g to the bottom of a 250 mL Duran® bottle. Analyse as per the procedure detailed on page 6, with the modification that a dilution of 100-fold is employed (i.e. dilute 1.0 mL of supernatant to 100.0 mL with 100 mM sodium maleate buffer, pH 6.6) and the

extraction volume is 47.35 mL (45.35 + 2.00). If dry weight values are required, determine the moisture content on a representative sample of the material.

(iv) Determination of available carbohydrates in carbonated drinks, wine, juices, mayonnaise and salad dressings.

Transfer 5 mL of solution to the bottom of a 250 mL Duran® bottle. Analyse as per the procedure detailed on page 6. Final extraction volume is 50.35 mL (the volume of buffer and enzymes, plus the volume of the sample, i.e. 45.35 + 5.0 mL). Allow for this in the calculations. If dry weight values are required, determine the moisture content of a representative sample of the material.

Table 1. **Total dietary fiber content of selected samples determined using AOAC method 991.43 (at ~ 100°C) and the currently described modified procedure (at 80°C).**

Sample	Total Dietary Fiber (dwb)[a]	
	AOAC 991.43	New Method[b]
Processed peas	18.0	19.9
Potato	7.0	7.4
Onion	17.0	17.1
Swede	29.1	29.9
Mushroom	21.0	22.1
Carrots	27.5	27.3
Baby corn	26.3	26.4
Lupin fiber	86.3	88.1
Potato fiber	67.1	64.1
Sugar beet pectin	88.1	93.3
Apple pectin	89.4	91.0
Ripe banana	6.8	8.7
Apple (Golden Delic.)	11.6	12.2
Spaghetti	6.1	6.6
Baked beans	17.9	18.6
Wheat germ	13.2	13.0
Wheat bran	53.3	53.5
Cracked wheat	6.7	6.0
Rice (long grain)	1.0	1.1
Egg noodle	4.6	4.8
Alpen Muesli™	8.3	8.4
Kellogg All Bran™	26.6	26.6
Weetabix™	10.5	10.7
White bread	4.2	4.5

[a] dry weight basis.

[b] DF as measured in the currently described procedure.

Table 2. **D-Fructose, D-glucose and available carbohydrate content of selected samples determined using the currently described procedure.**

Sample	Moisture % (w/w)	Carbohydrate Content "as is" basis, % (w/w)		
		D-Glu	D-Fru	ACH
Strawberries	91.9	2.1	2.3	4.4
Raspberries	89.2	1.4	1.6	3.0
Blackberries	79.2	4.9	4.9	9.8
Green grapes	78.3	9.8	9.6	19.4
Apple	86.3	3.1	7.7	10.8
Oranges	86.5	3.9	4.0	7.9
Grapefruit	86.9	3.0	3.1	6.1
Ripe banana	75.8	9.7	7.1	16.8
Green banana	74.7	8.1	0.0	8.1
Cantelope melon	90.0	3.9	4.1	8.0
Sweet corn	81.3	8.4	3.4	11.8
Baby corn (whole)	90.5	2.3	1.7	4.0
Sugar snap peas	86.8	5.8	1.6	7.4
Tomato	95.0	0.9	1.1	2.0
Red pepper	92.1	1.2	1.8	3.0
Brown onion	89.1	2.9	3.0	5.9
Potato	78.7	17.6	1.3	18.9
Broccoli	88.4	0.9	0.6	1.5
Swede	89.6	3.1	2.0	5.1
Turnip	84.1	5.1	3.5	8.6
Carrot	89.0	2.9	3.0	5.9
Mushroom	92.3	0.2	0.1	0.3
White bread	**42.2**	**46.0**	**0.0**	46.0
Bran bread	**39.0**	**36.2**	**0.6**	36.8
Digestive biscuits	**2.0**	**62.7**	**9.0**	71.7
Malted milk bisc.	**2.0**	**62.5**	**10.3**	72.8
Water crackers	**3.8**	**69.7**	**1.7**	71.4
Ryvita crackers	**4.8**	**71.7**	**0.8**	72.5
Weetabix™	**4.8**	**70.2**	**2.7**	72.9
Rice crispies	**2.0**	**93.5**	**1.7**	95.2
Muesli (Alpen™)	**7.4**	**74.1**	**9.4**	83.5
All Bran™	**1.0**	**43.4**	**8.3**	51.7
Rice cakes	2.9	88.7	0.0	88.7
Mars™ bar	**46.3**	**16.7**	**63.0**	79.7

* values in bold type were measured experimentally, while those in non-bold type were determined from the measured "dry weight" values shown in table 3.

Table 3. **D-Fructose, D-glucose and available carbohydrate content of selected samples determined using the currently described procedure.**

Sample	Moisture % (w/w)	Carbohydrate Content "dry weight" basis, % (w/w)		
		D-Glu	D-Fru	ACH
Strawberries	**91.9**	**26.5**	**28.8**	55.3
Raspberries	**89.2**	**13.0**	**14.7**	27.7
Blackberries	**79.2**	**23.4**	**23.5**	46.9
Green grapes	**78.3**	**45.2**	**44.4**	89.6
Apple	**86.3**	**22.4**	**56.2**	78.6
Oranges	**86.5**	**28.6**	**29.5**	58.1
Grapefruit	**86.9**	**22.8**	**23.5**	46.3
Ripe banana	**75.8**	**40.3**	**29.5**	69.8
Green banana	**74.7**	**31.9**	**0.0**	31.9
Cantelope melon	**90.0**	**39.4**	**40.8**	80.2
Sweet corn	**81.3**	**44.7**	**18.3**	63.0
Baby corn (whole)	**90.5**	**24.5**	**17.7**	42.2
Sugar snap peas	**86.8**	**43.6**	**12.1**	55.7
Tomato	**95.0**	**18.7**	**22.9**	41.6
Red pepper	**92.1**	**15.4**	**23.3**	38.7
Brown onion	**89.1**	**26.2**	**27.7**	53.9
Potato	**78.7**	**82.9**	**0.6**	83.5
Broccoli	**88.4**	**7.8**	**5.2**	13.0
Swede	**89.6**	**30.0**	**19.3**	49.3
Turnip	**84.1**	**31.9**	**21.7**	53.6
Carrot	**89.0**	**26.5**	**27.6**	54.1
Mushroom	**92.3**	**3.1**	**0.7**	3.8
White bread	**42.2**	**79.5**	**0.0**	79.5
Bran bread	**39.0**	**59.4**	**1.0**	60.4
Digestive biscuits	**2.0**	64.0	9.2	73.2
Malted milk bisc.	**2.0**	63.8	10.5	74.3
Water crackers	**3.8**	72.5	1.8	74.3
Ryvita crackers	**4.8**	75.3	0.8	76.1
Weetabix™	**4.8**	73.7	2.8	76.5
Rice crispies	**2.0**	95.4	1.7	97.1
Muesli (Alpen™)	**7.4**	80.0	10.2	90.2
All Bran™	**1.0**	43.8	8.4	52.2
Rice cakes	**2.9**	**91.4**	**0.0**	91.4

* values in bold type were measured experimentally, while those in non-bold type were determined from the measured "as is" values as shown in table 2.

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