

1.12979.0001

Total Dietary Fiber

Reagent kit for 100 determinations (double determinations for max. 50 samples)
(Refer to L.00.00-18 § 35 LMBG, January 1997)

Method of determination

Enzymatic-gravimetric method for the determination of Total Dietary Fiber in foods.

Contents of package and storage

α -Amylase solution	5 ml
Protease solution	5 ml
Amyloglucosidase solution	3 × 5 ml

Store the reagents at +2 to +8°C.

General

The term "Total Dietary Fiber" covers a large number of complex organic compounds, essentially vegetable, non-starch polysaccharides and lignin. These substances (cellulose, hemicelluloses, pectins, hydrocolloids, resistant starch and lignin) are not attacked by the human digestive enzymes. A healthy diet should include a sufficient amount of dietary fiber; a knowledge of the dietary fiber contents of foods is therefore necessary. The method described here for the determination of total dietary fiber is an improvement of the method described by Prosky et al.

Lee, S.C., Prosky, L., de Vries, J.W.: Determination of total, soluble and insoluble dietary fiber in foods; enzymatic-gravimetric method. MES-TRIS buffer: collaborative study, Journal of AOAC International **75** (3), 395 - 416 (1992).

Short description

Total Dietary Fiber uses a combination of enzymatic and gravimetric methods (Asp, N.G., Johansson, C.G., Hallmer, H., and Siljeström, M., Rapid Enzymatic Assay of Insoluble and Soluble Dietary Fiber, J. Agric. Food Chem., 1983, 31, 476 - 482, and: Official Methods of Analysis of the Assoc. of Anal. Chem. 1984, 47.021 - 47.027, 14th edition). The investigation must always be carried out using two samples that differ very little in weight. The sample material is first treated with heat-resistant α -amylase in order to convert the starch to a paste and achieve its partial breakdown. This is followed by protein digestion with protease and breakdown of the residual starch with amyloglucosidase. The soluble dietary fiber is precipitated with ethanol (95 % volume concentration) and the precipitate is filtered off and washed with ethanol and acetone. The residue is then dried and weighed. The protein content of the residue of the first sample batch is determined by the Kjeldahl method, and the ash content of the residue of the second batch is determined. The mean weight of the two residues which is obtained after subtraction of the values for protein, ash and blank solution corresponds to the content of dietary fiber in the product.

Reagents

Contained in the pack:

α -Amylase solution
Protease solution
Amyloglucosidase solution

Not contained in the pack:

Petroleum benzine GR, boiling range 40 - 60°C

Water, distilled

Ethanol (95 %, v/v)

prepared from 95 ml ethanol absolute + 5 ml distilled water

Ethanol (78 %, v/v)

prepared from 78 ml ethanol absolute + 22 ml distilled water

MES/TRIS buffer solution c = 0.05 mol/l, pH 8.3

Dissolve 2.13 g MES (2-morpholinoethansulfonic acid monohydrate) and 1.22 g TRIS (tris(hydroxymethyl)-aminomethane) in approx. 170 ml distilled water and adjust to pH 8.3 at 20 °C with sodium hydroxide solution 6 mol/l. Then make up with distilled water to 200 ml.

Sodium hydroxide solution (6 mol/l)

Dissolve 24 g NaOH in 70 ml distilled water, make up to 100 ml.

Sodium hydroxide solution, 5 %

Dissolve 6.8 g NaOH in 70 ml distilled water, make up to 100 ml.

Hydrochloric acid (0.56 mol/l)

Make up 28 ml hydrochloric acid 2 mol/l with distilled water to 100 ml.

Hydrochloric acid, 5 %

Make up 80 ml hydrochloric acid 2 mol/l with distilled water to 100 ml.

Acetone, GR

Celite® 545, acid washed

Equipment and sundries

Analytical balance

accuracy 0.1 mg

Filter crucible (glass crucible with frit)

diameter 40 mm

porosity 40 - 90 µm (G2)

For preparation of the crucible, see "Determination procedure", section 2

Drying cabinet (105 °C)

Desiccator with drying agent

Glass beakers (400 ml, 250 ml, 600 ml)

Waterbath with thermostat, 60 °C and 100 °C, equipped with magnetic stirrer

pH meter

Plunger pipettes, 50 µl and 150 µl

Wash bottle or filter apparatus acc. to Witt

Vacuum pump (water jet pump)

Muffle furnace, adjustable to 525 °C

Mortar or laboratory screen-type mill

Watch glasses, rubber squeegee, glass rods, magnetic stirring rods, filter adapters, etc.

Sample preparation

Using a laboratory screen-type mill, grind samples with particle sizes > 0.3 mm which do not dissolve in water or cannot be ground in the mortar. Foods with a high water content must be homogenized before milling as follows. Dry overnight at 105 °C in a drying cabinet and cool in a desiccator. Then homogenize by sonication or using a blender. Subsequently mill (particle size 0.3 - 0.5 mm mesh). If the sample cannot be heated, it must be freeze-dried before milling.

Removal of fat

If the fat content exceeds 5 %, fat must be removed with petroleum benzene before the sample is ground, as follows: For each g of sample (accurately weighed) extract with 3 × 25 ml of petroleum benzene. Dry the sample remaining in the beaker in a drying cabinet at 70 °C under vacuum, then weigh and grind. Allowance must be made for weight loss during calculation of the total dietary fiber content.

Determination procedure

1. Enzymatic breakdown

Determine each sample and blank in duplicate:

- Weigh 1.0 g of sample (to the nearest 0.1 mg) into two 400-ml beakers (250-ml beakers for determination of soluble and insoluble dietary fiber) and also prepare two blanks (without sample). The difference in weight between paired sample quantities must not exceed 20 mg. In the case of high-fiber products such as bran, the amount of sample taken can be reduced. In the case of samples with a high water content, use a quantity equivalent to 1 g dry weight.
- Add 40 ml MES/TRIS buffer solution to each beaker. In the case of acidic products such as rye bread or fruit, check the pH and adjust where necessary to pH 8.3 with sodium hydroxide solution (5 %). During subsequent enzymatic breakdown, constantly agitate the beakers and cover with watch glasses. Incubate in a water bath.
- Add 50 µl α-amylase solution, incubate for 30 min at 95 - 100°C and cool to 60 °C.
- Add 50 µl protease solution and incubate for 30 min at 60 °C.
- Add 5 ml hydrochloric acid (0.56 mol/l) and adjust at 60 °C to pH 4.0 - 4.7 with sodium hydroxide solution 5 % or hydrochloric acid 5 %.
- Add 150 µl amyloglucosidase solution and incubate for 30 min at 60 °C.

2. Determination of Total Dietary Fiber

- Following enzymatic breakdown heat 220 ml ethanol 95 % to 60 °C and add to the contents of each beaker. Allow the precipitate to settle for at least 1 h at room temperature and then, decanting under slight vacuum, filter through the prepared glass filter crucibles (wash residues remaining in the beaker into the crucible with small amounts of ethanol 78 %). Prepare the glass filter crucibles used as follows: Heat the thoroughly cleaned glass crucibles for 1 h at 525 °C, add approximately 1 g Celite® 545 (ignited overnight at 525 °C, cooled, and stored in a stoppered container), dry overnight at 105 °C, cool and weigh to the nearest 0.1 mg. In order to avoid negative blank values, new crucibles will need to be prepared repeatedly as described. Crucibles should be cooled and stored in a desiccator until required.
- Wash the residues with 3 × 15 ml ethanol 78 %, then rewash with 2 × 10 ml ethanol 95 % and 3 × 10 ml acetone.
- Dry overnight at 105 °C.
- Cool and weigh to the nearest 0.1 mg.
- When determining protein and ash, use separate filter crucibles for sample and blank.

3. Determination of soluble and insoluble dietary fiber separately

3.1 Determination of insoluble dietary fiber

- Following enzymatic breakdown filter the liquid in the beakers under slight vacuum through the prepared glass filter crucibles.
- Rinse the beakers and residues with 2 × 10 ml water heated to 70 °C.
- Combine the filtrate and rinsings and transfer to tared 600-ml glass beakers.
- Rinse the residues with 2 × 15 ml ethanol 78 %, ethanol 95 % and acetone.
- Dry overnight at 105 °C.
- Cool and weigh to the nearest 0.1 mg.
- When determining protein and ash, use separate filter crucibles for sample and blank.

3.2 Determination of soluble dietary fiber

- Weigh the combined filtrates and rinsings in the tared beakers to the nearest 0.1 g and add 4 times the quantity of ethanol 95 % previously heated to 60 °C. Use an aliquot of the hot ethanol to rinse the suction flask used in the determination of insoluble dietary fiber. Allow the precipitate that forms to settle for at least 1 h at room temperature and then, decanting under slight vacuum, filter through the prepared glass filter crucibles (wash residues remaining in the beaker into the crucible with small amounts of ethanol 78 %).
- Wash the residues with 3 × 15 ml ethanol 78 %, then rewash with 2 × 10 ml ethanol 95 % and 3 × 10 ml acetone.
- Dry overnight at 105°C.
- Cool and weigh to the nearest 0.1 mg.
- When determining protein and ash, use separate filter crucibles for sample and blank.

Notes: Settling of the precipitate in the determination of total dietary fiber (section 2) and of soluble dietary fiber (section 3.2) can be facilitated by maintaining the beakers at 45 °C (1 h) in a water bath. With some products a tacky residue may form, which will slow down filtration. Filtration can be speeded up again by using a spatula to carefully break the surface of the residue and move it to one side.

4. Determination of protein and ash

4.1 Determination of protein

- Transfer the entire residue of one filter crucible to a Kjeldahl flask and determine nitrogen by the Kjeldahl method.
- Calculation:
$$\text{mg protein} = \text{mg nitrogen} \times 6.25$$

4.2 Determination of ash content

- Ignite the residue in the filter crucible at 525 °C for 5 hours, in a muffle furnace.
- Cool, weigh to the nearest 0.1 mg, and calculate the ash content in mg.
If negative ash values encountered, treat the crucibles several times as described in section 2. Nevertheless, negative values are still to be used in calculations.

5. Calculation

Dietary fiber content w in %

$$w = \frac{m_R - m_P - m_A - m_B}{m} \times 100$$

$$w = \frac{m_R - [(V_1 - V_2) \times 1.4007 \times 6.25] - m_A - [m_{RBlank} - [(V_1 - V_{2Blank}) \times 1.4007 \times 6.25] - m_{ABlack}]}{m} \times 100$$

$$m_B = m_{RBlank} - m_{PBlank} - m_{ABlack}$$

$$m_P = (V_1 - V_2) \times 1.4007 \times 6.25$$

w: total dietary fiber, by weight, in %

m_B: mass of the blank, in mg

m_P: mass of protein in the residue, in mg

m_R: mean of residue masses, in mg

m_A: mass of ash in the residue, in mg

V₁: amount of HCl 0.1 mol/l measured out, in ml

V₂: amount of NaOH 0.1 mol/l consumed, in ml

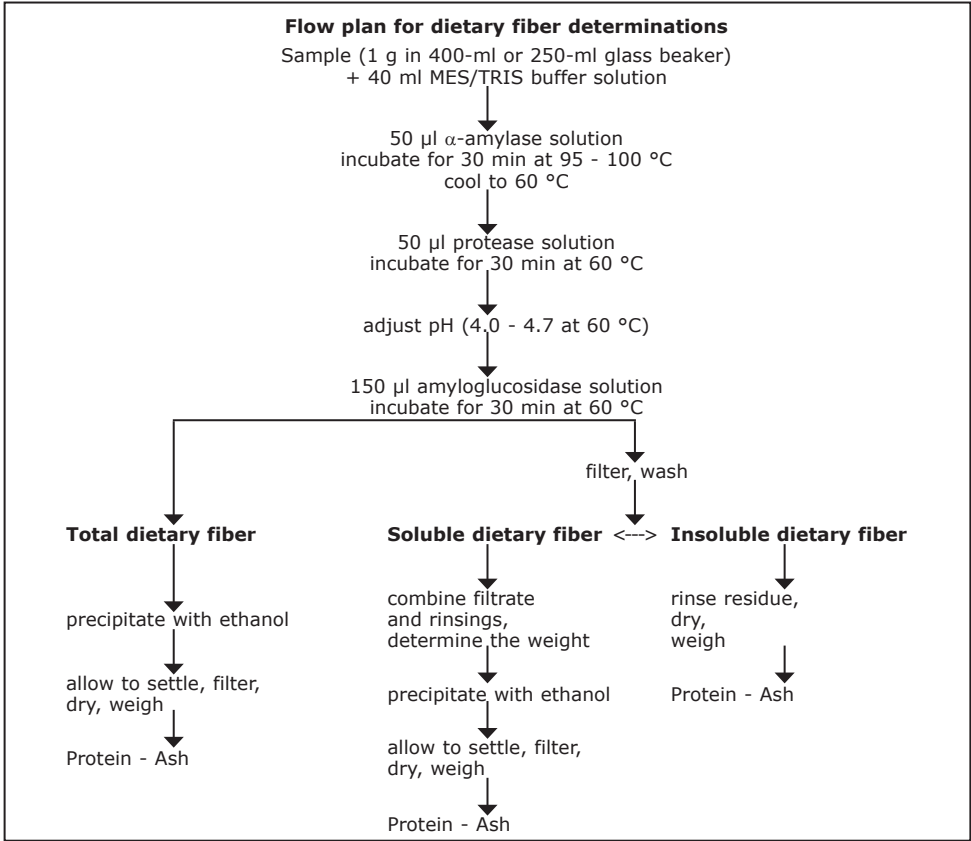
m: mean of the sample weights, in mg

Use the equation set out above to calculate dietary fiber. Enter values as follows:

for **total dietary fiber content** the results from section 2,

for **insoluble dietary fiber** the results from section 3.1,

for **soluble dietary fiber** the results from section 3.2.



Typical values

Sample	Total dietary fiber content [%]
Bio muesli	9.4
Wholemeal bread	11.9
Crispbread	12.0 - 13.7
Rolled oats	10.2

Information for ordering reagents

Cat. No.	Designation	Package sizes
101775	Petroleum benzine for analysis EMSURE®	1 l, 2.5 l, 5 l
100983	Ethanol, absolute for analysis EMSURE®	1 l, 2.5 l, 5 l
108382	Tris(hydroxymethyl)aminomethane GR for analysis, buffer substance	100 g, 500 g, 1 kg
106126	2-Morpholinoethanesulfonic acid monohydrat, buffer substance MES	25 g, 250 g, 1 kg
106498	Sodium hydroxide pellets for analysis EMSURE®	500 g, 1 kg, 5 kg
109063	Hydrochloric acid 2 mol/l (2 N) Titripur®	1 l
102693	Celite® 545	250 g, 1 kg
100014	Acetone for analysis EMSURE®	1 l, 2.5 l
112979	Total Dietary Fiber	1 pack

