

MAFF VALIDATED METHODS FOR THE ANALYSIS OF FOODSTUFFS

No. V 1

DIETARY FIBRE (COLORIMETRY)

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1 Scope and field of application

The method determines dietary fibre as non-starch polysaccharides (NSP) and as NSP plus resistant starch (RS).

2 Definition

Content of dietary fibre: the content of NSP, or NSP plus RS, as determined by the method specified.

3 Principle

The starch is gelatinised and then removed by enzymic digestion. The remaining polysaccharides are hydrolysed by sulphuric acid and the resulting sugars determined colorimetrically. Two alternative procedures are described for the dispersion of the starch thus enabling evaluation of the samples for dietary fibre as NSP alone and as NSP plus RS. The procedures are summarised in Appendix 1.

4 Reagents

High purity reagents are used throughout the method. Distilled water, or water of an equivalent purity, is to be used.

4.1 Acetone

4.2 Dimethyl sulphoxide

4.3 Benzoic acid, 50% saturated: prepare with saturated benzoic acid and water diluted 1 litre + 1 litre.

4.4 Sodium acetate buffer, 0.1 mol/l, pH 5.2

4.4.1 Sodium acetate, 0.1 mol/l: prepare by dissolving 13.6 g sodium acetate trihydrate, $\text{CH}_3\text{COONa} \cdot 3\text{H}_2\text{O}$, and making up to 1 litre with benzoic acid solution (4.3).

4.4.2 Acetic acid, 0.1 mol/l.

4.4.3 Calcium chloride, 1 mol/l.

4.4.4 Sodium acetate buffer, 0.1 mol/l: adjust sodium acetate 0.1 mol/l (4.4.1) to pH 5.2 with acetic acid, 0.1 mol/l (4.4.2). To stabilise and activate enzymes, add 4 ml of calcium chloride, 1 mol/l (4.4.3) to 1 litre of buffer.

- 4.5 α -Amylase, EC 32.1.1: Pancrex V Capsules (approximately 9000 BP units α -amylase per capsule, Paines and Bryne Ltd).
- 4.5.1 α -Amylase solution, 2000 BP units/ml: empty 2 Pancrex V capsules (4.5) into a centrifuge tube. Add 9 ml of water and disperse using a vortex mixer. Centrifuge at 1500 g for 10 min. and use the supernatant as α -amylase solution. Prepare immediately before use.
- 4.6 Pullulanase, 100 units/ml: EC 3.2.1.41 (Boehringer 108944)
- 4.6.1 Pullulanase solution, 1 unit/ml: dilute pullulanase (4.6) 1:100 (eg 0.010 ml made up to final volume of 1 ml) with acetate buffer (4.4.4). Prepare immediately before use.
- 4.7 Ethanol, absolute
- 4.8 Ethanol, 85% (V/V).
- 4.9 Sulphuric acid, 12 mol/l.
- 4.10 Sulphuric acid, 2 mol/l.
- 4.11 Glucose solution, 0.5 mg/ml: in 50% saturated benzoic acid solution (4.3).
- 4.12 Sodium hydroxide
- 4.13 Sodium hydroxide solution, 3.9 mol/l.
- 4.14 Sodium potassium tartrate
- 4.15 Dinitrosalicylate solution: dissolve 10 g of 3,5-dinitrosalicylic acid, 16 g of sodium hydroxide (4.12) and 300 g of sodium potassium tartrate (4.14) in re-distilled water and make up to 1 litre with this water. Store in a well-capped dark bottle. Keep for 2 days before use. The solution is stable for at least six months at room temperature.
- 4.16 Standard sugar solution: dissolve 600 mg of arabinose, 800 mg of xylose and 600 mg glucose in benzoic acid solution (4.3) and make up to 500 ml with the benzoic acid solution to provide a stock solution. To prepare standards, take 1, 2, 3 and 4 ml of stock solution and make up to 4 ml with the benzoic acid. Add 4 ml of sulphuric acid, 2 mol/l (4.10), to give standards of 0.5, 1.0, 1.5 and 2.0 mg total sugars/ml in sulphuric acid 1 mol/l.

5 Apparatus:

- 5.1 Centrifuge
- 5.2 Centrifuge tubes: glass centrifuge tubes of 50-60 ml capacity, fitted with screw-tops.
- 5.3 Hot plate and stirrer: Place a beaker of water on the magnetic stirrer/hot plate and bring it to the required temperature. Use a beaker of sufficient capacity and height to contain the required number of tubes, 'e.g. 12.' Place a layer of Scotch-Brite or similar material in the bottom of the beaker to prevent breakage. Cover the beaker, e.g. with tinfoil, to aid boiling and ensure even temperature distribution.
- 5.4 Magnetic stirrers: PTFE coated, to fit centrifuge tubes (5.2).
- 5.5 Vortex mixer

- 5.6 Water-bath: this should be of such capacity that there is no significant change in temperature when a rack containing all the tubes, samples and standards is placed in it.
- 5.7 Spectrophotometer, capable of measurement at 530 nm.
- 5.8 Oven, capable of maintaining a temperature of $42 \pm 2^\circ\text{C}$ for 24 hr.

6 Procedure

6.1 Pre-treatment of samples

Foods are analysed without pretreatment whenever possible. If there are problems in taking a representative sample, foods with a low water content may be milled and foods with a higher water content may be homogenised or freeze-dried and milled. No sample should be subjected to ball-milling for more than 2-3 min.

6.2 Test samples

Accurately weigh, to the nearest 0.1 mg, two portions (a) and (b) of the sample, each between 100 and 500 mg (but containing no more than 200 mg of dry matter and 50 mg NSP, e.g. 200 mg flour, 100 mg bran) into a 50 ml screw-top centrifuge tube (5.2) and add a stirrer (5.4).

Portion (a) in tube (a) will be used to measure the total NSP content of the sample: portion (b) in tube (b) will be used to measure total NSP together with RS. These methods should be considered to be alternatives, so normally only tube (a) or tube (b) will be needed.

6.3 Fat extraction

Dry samples (i.e. 90-100% dry matter) with less than 5% fat may be analysed directly. Otherwise add 40 ml acetone (4.1), mix for 30 min. using a magnetic stirrer, centrifuge and remove by aspiration as much of the supernatant as possible without disturbing the residue and dry it with stirring at $62.5 \pm 2.5^\circ\text{C}$ (6.4.2).

6.4 Dispersion and enzymic hydrolysis

6.4.1 Dispersion of the starch

Add 2 ml of DMSO (4.2) to tube (a) and mix for about 2 min. at room temperature, using a magnetic stirrer. Add 10 ml of acetate buffer (4.4.4) to tube (b). Cap and place tubes (a) and (b) in the beaker with boiling water (5.3) for 1 to 1.25 hr. timed from when re-boiling commences. Use the magnetic stirrer (note that in tube (a) gel formation may occur to such an extent that the stirrer is prevented from moving, but this will not affect the procedure).

Remove tube (a) from the beaker and immediately, without cooling, add 8 ml of acetate buffer (4.4.4), pre-equilibrated at $50 \pm 5^\circ\text{C}$, and vortex mix. Then remove tube (b) from the beaker.

6.4.2 Enzymic hydrolysis of starch

Note: All portions of the sample in their associated tubes are to be treated in the same manner in the procedure given in Section 6.4.2 through to Section 6.6 of the method. Reference to "a sample" or "a

tube" is taken to include "all samples" or all tubes" being analysed in this part of the method.

Leave the tubes at room temperature or in a water bath at 37°C, but only until the contents have cooled to between 30°C and 40°C and then immediately add 0.5 ml α -amylase solution (4.5.1) followed by 0.1 ml of pullulanase solution (4.6.1) and vortex-mix. (NB: do not combine the enzyme solutions before addition).

Incubate the sample at $42 \pm 2^\circ\text{C}$ (5.6 or 5.8) for 16 to 18 hr. Vortex mix after the first hour.

After the enzyme treatment add 40 ml of absolute ethanol (4.7) to each tube, mix well by inversion and leave for 1 hr. at room temperature. Centrifuge at 1500 g for 10 min. or until a clear supernatant liquid is obtained.

Remove by aspiration as much of the supernatant as possible without disturbing the residue and discard it. Wash the residue twice with 85% ethanol (4.8), using 50 ml each time. Mix by inversion and then use a magnetic stirrer to form a suspension of the residue (about 5 to 10 min.), centrifuge until clear and remove the supernatant by aspiration as before.

Add 40 ml of acetone (4.1) to the washed residue, stir magnetically for 5 min. and then centrifuge at 1500g for approximately 10 min. or until clear. Remove the supernatant by aspiration and discard it. Place the tube in a beaker of water at $62.5 \pm 2.5^\circ\text{C}$ on the hot plate stirrer (5.3) and mix the residue for a few minutes until it appears dry. The beaker may be covered and the acetone vapour removed by a water pump.

6.5 Acid hydrolysis of the residue from enzymic digestion

Disperse the dried residue from Section 6.4.2 in 2 ml of sulphuric acid, 12 mol/l (4.9) with vortex-mixing. Leave at $35 \pm 1^\circ\text{C}$ (5.6) for 1 to 1.25 hr., with occasional mixing to disperse the cellulose. Rapidly add 22 ml of water and mix. Place in boiling water (5.3) for 2 to 2.25 hr. from re-boiling, stirring continuously. Cool to room temperature.

6.6 Measurement of total reducing sugars

Place into separate test tube 1 ml of blank solution (Benzoic acid solution (4.3) and sulphuric acid solution, 2 mol/l, (4.10) diluted 0.5 ml + 0.5 ml), 1 ml of each of the standard solutions (4.16) and 1 ml of the hydrolysate (6.5). Add 0.5 ml glucose solution, 0.5 mg/ml (4.11) and 0.5 ml of sodium hydroxide, 3.9 mol/l (4.13) to each tube and vortex-mix.

Add 2 ml of dinitrosalicylate solution (4.14) to each tube and vortex-mix again.

Place the tubes, all at the same time, in a briskly boiling water-bath (5.6) for 10 min. Cool in water to room temperature. Add 20 ml of water and mix well by repeated inversion. Read the absorbance at 530 nm.

7 COSHH

Analysts are reminded that appropriate hazard and risk assessments required by the Control of Substances Hazardous to Health Regulations, 1988 (See "Control of Substances Hazardous to Health - Approved Code of Practice, Control of Substances Hazardous to Health Regulations 1988") must be made before using this method.

8 Expression of Results

8.1 Calculation of non-starch polysaccharides

The NSP content, in g/100g, is given by:

$$\text{NSP (g/100g)} = \frac{A_t}{A_s} \times \frac{V_t}{W_t} \times 100$$

where:

- A_t is the absorbance of the test solution obtained from tube (a);
- V_t is the total volume (ml) of the test solution (here 24 ml);
- A_s is the absorbance corresponding to 1 mg sugar/ml taken from the line of best fit for the standard; and
- W_t is the weight (mg) of sample taken for analysis in tube (a).

8.2 Calculation of non-starch polysaccharides with resistant starch

The NSP content together with resistant starch, in g/100g is given by:

$$\text{NSP+RS (g/100g)} = \frac{A_t}{A_s} \times \frac{V_t}{W_t} \times 100$$

where:

- A_t is the absorbance of the test solution obtained from tube (b);
- V_t is the total volume (ml) of the test solution (here 24 ml);
- A_s is the absorbance corresponding to 1 mg sugar/ml taken from the line of best fit for the standard; and
- W_t is the weight (mg) of sample taken for analysis in tube (b).

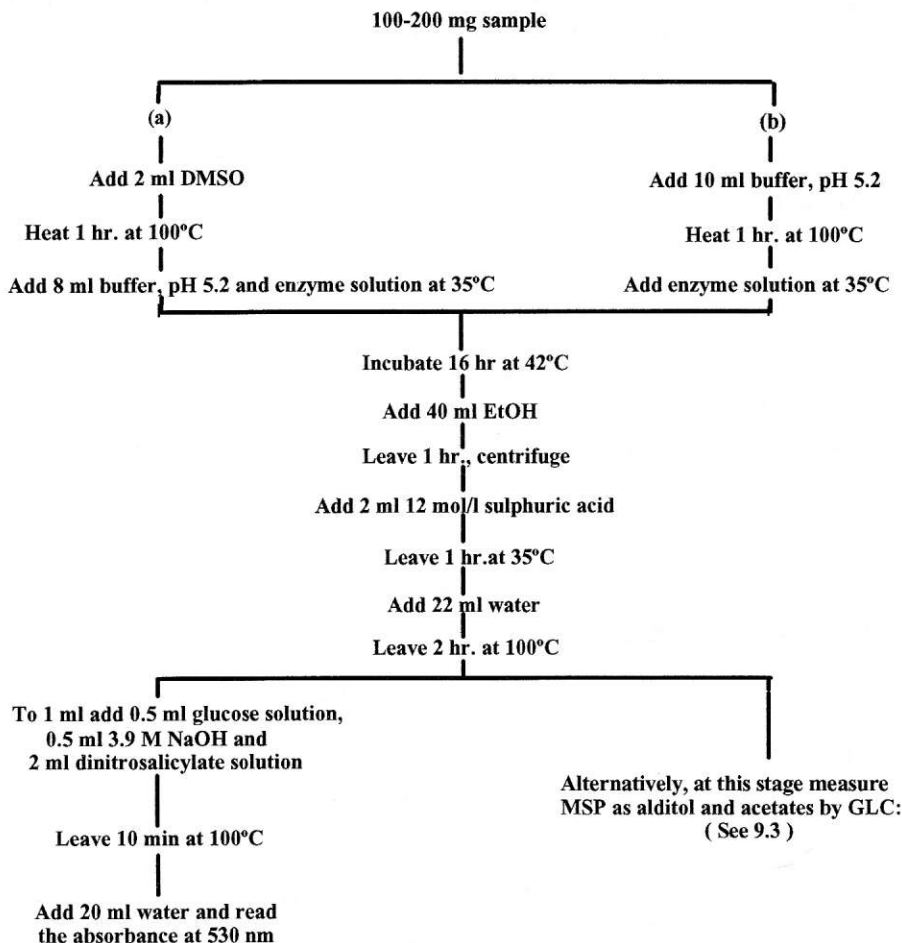
9 References

- 9.1 H N Englyst, J H Cummings and R Wood J.Assoc. Publ. Analysts, 1987, **25**, 73-110
- 9.2 H N Englyst and G J Hudson. Food Chem., 1987,**24**,63-76.
- 9.3 MAFF Validated Methods for the Analysis of Foodstuffs. No V2; Dietary Fibre, Englyst Method: GLC End-point Determination. London, 1990.

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APPENDIX 1

SCHEMATIC DIAGRAM FOR ANALYSIS OF DIETARY FIBRE



APPENDIX 2

Analytical Quality Control

General principles of analytical quality control are outlined in protocol V0 of the series.

A1 Repeatability

The absolute difference between two test results obtained under repeatability conditions should not be greater than the repeatability, r , deduced from the collaborative trial data summarised in Tables 1 and 2. Although these do not show a consistent pattern among samples with different matrices and fibre contents, r should normally be taken as 2 g/100g.

A2 Reproducibility

The absolute differences between two test results carried out under reproducibility conditions should not be greater than the reproducibility, R , deduced from the collaborative trial data below (Tables 1 and 2). Although these do not show a consistent pattern among samples with different matrices and fibre contents, R should normally be taken as 4 g/100g.

A3 Trueness (Bias)

The trueness of the results cannot be estimated directly in the absence of pure analyte and of reference materials. Comparison of mean values from wholemeal and white breads and a mixture of the two indicates consistent recoveries during the collaborative trial. All the mean values of NSP and NSP plus RS observed during the trial agree quantitatively with those obtained by the full Englyst procedure (9.3), which may be regarded as a reference procedure only because it gives more detailed information of the specific sugar composition of the dietary fibre, after analysis by GLC. In this sense the full and simplified procedures are both accurate in the determination of dietary fibre as defined in Section 2 of this protocol, but observed values do not necessarily agree with results using other methods and other types of sample. In particular, they may not agree with the true levels of polysaccharide that escapes assimilation by an animal during digestion, though they are designed to reflect this. It is recommended that analytical data should normally be based on NSP alone, since RS is usually a minor constituent, it depends on the processing history of the sample and its physiological significance is uncertain.

The determination of RS alone, by subtracting NSP from NSP + RS (calculated as in Section 8.1 of the protocol) introduces excessive variability and cannot be recommended. The method is not precise enough to be suitable for the estimation by difference of the small levels of RS usually encountered.

A4 Limit of Detection

This limit has not been established. The collaborative trial data suggests an accuracy which, if maintained at low fibre levels, corresponds to a lower limit of roughly 1 g/100g for a duplicate determination.

A5 Statistical Data: Derived from Results of Interlaboratory Tests

Participants in the collaborative trial at 19 laboratories each analysed a sample of wholemeal bread in duplicate as a pre-trial check. They then each analysed 14 samples once in the trial proper. These comprised 7 different samples (including the same wholemeal bread) in blind duplicate. The samples had been commercially prepared, ground and homogenised; they were artificially coloured to appear identical.

Tables 1 and 2 summarise the statistical data; the dietary fibre levels were expressed as a percentage by mass of the sample on a dry weight basis, and $\bar{x}$ was the overall observed mean value.

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TABLE 1

Statistical Analysis of the % Non-starch Polysaccharide in Various Matrices

Sample	$\bar{x}$	S_r	RSD_r	r	S_R	RSD_R	R
Wholemeal bread (pre-trial sample)	10.59	0.46	4.4	1.29	1.07	10.1	3.00
Wholemeal bread	10.66	0.64	6.0	1.79	1.87	17.5	5.23
Wholemeal/white bread (1:1)	7.43	0.98	13.1	2.73	1.21	16.3	3.39
White bread	3.08	0.55	18.0	1.55	0.64	20.8	1.79
High bran bread	11.53	0.55	4.8	1.54	1.57	13.6	4.4
Rye bread	4.71	0.63	13.3	1.75	0.72	15.3	2.02
Cornflakes	1.09	0.33	29.8	0.91	0.41	37.4	1.14
Oat cereal	8.73	0.76	8.7	2.13	1.58	18.1	4.43

TABLE 2

Statistical Analysis of the % Non-starch Polysaccharide Plus Resistant Starch in Various Matrices

Sample	$\bar{x}$	S_r	RSD_r	r	S_R	RSD_R	R
Wholemeal bread (pre-trial sample)	11.48	0.43	3.7	1.29	0.96	8.4	2.69
Wholemeal bread	11.45	0.75	6.6	2.11	1.88	16.4	5.26
Wholemeal/white bread (1:1)	8.53	1.03	12.0	2.87	1.13	13.2	3.15
White bread	4.19	0.64	15.2	1.78	0.73	17.4	2.04
High bran bread	11.85	1.64	13.9	4.60	2.04	17.2	5.70
Rye bread	6.08	0.35	5.7	0.98	0.75	12.3	2.10
Cornflakes	3.89	0.61	15.8	1.72	0.70	18.1	1.97
Oat cereal	9.30	0.49	5.2	1.36	1.76	18.9	4.93

A6 Key to Tables 1 and 2

Symbol	Definition
$\bar{x}$	Overall mean value
S_r	The standard deviation of repeatability
RSD_r	The relative standard deviation of repeatability, expressed as a percentage of the mean (coefficient of variance of repeatability CV_r)
r	Repeatability
S_R	The standard deviation of reproducibility
RSD_R	The relative standard deviation of reproducibility, expressed as a percentage of the mean (coefficient of variance of reproducibility CV_R)
R	Reproducibility