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MAFF VALIDATED METHODS FOR THE ANALYSIS OF FOODSTUFFS

No. V 26

CRUDE FIBRE IN FLOURS

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1. Scope and Field of Application

The method allows the determination of crude fibre in bread and flour. It is applicable to samples that contain less than 2% of crude fibre, but higher contents can be accommodated.

2. Definition

Crude fibre content: the content of crude fibre as determined by the method specified.

3. Principle

The sample undergoes oxidative digestion, followed by oxidation of the residue in solution by potassium dichromate and determination of the excess dichromate by means of titration with thiosulphate.

4. Reagents

All reagents should be of recognised analytical grade unless specified otherwise. Wherever the use of water is required, distilled or water of equivalent purity is to be used.

4.1 Diethyl ether

4.2 Sulphuric acid, 96% *m/m*.

4.3 Scharrer-Kurschner reagent

Dissolve 25.0 g of trichloroacetic acid (**4.3.1**) and 62 ml of nitric acid (**4.3.2**) in acetic acid (**4.3.3**) and make up with the acetic acid (**4.3.3**) to 1 l.

4.3.1 Trichloroacetic acid

4.3.2 Nitric acid, 65% *m/m* (13.1 gram equivalent per kg).

4.3.3 Acetic acid, 70% *m/m* (11.7 gram equivalent per kg).

4.4 Potassium dichromate in sulphuric acid, approximately 0.033 mol/l. Dissolve 2.45 ± 0.05 g of potassium dichromate in 160 ± 5 ml of water and add 120 ± 5 ml of concentrated sulphuric acid (**4.2**).

4.4.1 Potassium dichromate

4.5 Potassium iodide solution, 1 mol/l. Dissolve 16.6 ± 0.5 g of potassium iodide in 100 ± 5 ml of water.

4.5.1 Potassium iodide

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4.6 Sodium thiosulphate solution, approximately 0.1 mol/l, standardised with a precision of 0.5%.

4.6.1 Sodium thiosulphate

4.7 Starch indicator

Add a suspension of 5 g of soluble starch in 30 ml of water to 1 litre of boiling water, and continue boiling for 3 min; cool and add, if desired, 10 mg of mercury(II) iodide as a preservative.

4.7.1 Soluble starch

4.7.2 Mercury(II) iodide

5. Apparatus

5.1 Digestion assembly, illustrated in Fig. 1 and consisting of the following parts.

5.1.1 Pear-shaped acetylation flasks, 150 ml, with ground glass joint NS 24/29 female.

Note that if the sample contains less than 0.2% crude fibre, larger flasks are necessary to accommodate the volume of diethyl ether required for the extraction.

5.1.2 Air condenser, with ground glass joint NS 24/29 male.

5.1.3 Argand burner

5.2 Extraction assembly, illustrated in Fig. 2 and consisting of the following parts.

5.2.1 Erlenmeyer suction flask, 500 ml, with the bottom cut off and ground edge to fit onto a glass plate ground flat; or a Witt filter chamber (e.g. Jencons) or equivalent apparatus.

5.2.2 All-glass Buchner funnel, with sintered glass disc filter of pore diameter 15-40 µm (e.g. 17G3 Schott-Jena), overall diameter 65 mm; to fit suction flask (**5.2.1**).

5.2.3 Erlenmeyer receiving flasks, 100 ml.

5.3 Erlenmeyer suction flasks, 500 ml.

5.4 Erlenmeyer flasks, 1 l, with wide necks and ground glass stoppers.

6. Procedure

6.1 Sample preparation

Grind the sample so that it passes completely through a 1 mm sieve.

6.2 Digestion

Weigh, to the nearest 1 mg, an amount of sample that is expected to contain between 5 and 25 mg of crude fibre. Transfer to an acetylation flask (**5.1.1**). Add Scharrer-Kurschner reagent (**4.3**), 15 ml per g of test sample, but not less than 10 ml. Attach the air condenser (**5.1.2**). Bring the contents of the flask to the boil, and boil gently for 60 ± 1 min, swirling periodically. Cool to room temperature.

6.3 Extraction

Add diethyl ether, 1.5 times the volume of Scharrer-Kurschner reagent used, swirl for a few seconds and filter the top layer only through the Buchner funnel (**5.2.2**) into a suction flask (**5.3**). Repeat the extraction twice with the same volume of diethyl ether, and reject the filtrate. Add 25 ml of cold reagent (**4.3**) to the acetylation flask. Apply suction to the suction flask and pour the entire contents of the acetylation flask directly onto the frit of the Buchner funnel. Transfer and wash the collected residue with cold reagent (**4.3**) until the filtrate no longer shows cloudiness on the addition of water; usually washing 3 times with 10 ml is sufficient. Remove the filtrate from the suction flask. Place the Buchner funnel on it again, wash with hot water until acid-free to litmus; usually 3-5 times is sufficient.

6.4 Dissolution

Pipette 25 ml of potassium dichromate solution (**4.4**) into an Erlenmeyer receiving flask (**5.2.3**) and place it in the modified suction flask (**5.2.1**). Fit the Buchner funnel with the washed residue after extraction to the suction flask (**Fig. 2**). Place 5 ml of sulphuric acid (**4.2**) on the filter and stir until the residue has dissolved completely. Draw off the acid solution into the receiving flask by suction. Wash five more times with 5 ml of sulphuric acid, each portion always being completely transferred before the next portion is added.

6.5 Oxidation

Take the Erlenmeyer receiving flask with the solution of crude fibre in dichromate-sulphuric acid out of the suction flask. Place it in a boiling water bath for 10 min.

6.6 Titration

After cooling, wash the contents of the Erlenmeyer receiving flask with 500 ml of water into an Erlenmeyer flask (**5.4**). Cool again; then pipette, while swirling, 10 ml of potassium iodide solution (**4.5**) into the flask and close it. After standing it for 10 min in the dark, titrate with sodium thiosulphate solution (**4.6**), using starch (**4.7**) as indicator.

6.7 Blank titration

Carry out a blank titration with 25 ml of potassium dichromate, 50 ml of water, 30 ml of sulphuric acid and 10 ml of potassium iodide.

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

The content of crude fibre, calculated as a percentage by mass of the prepared sample on a dry basis, is given by:

$$\% \text{ crude fibre} = 0.686 \times \frac{(V_2 - V_1) \times T}{m} \times \frac{100}{100 - H}$$

Where:

V_1 is the volume, in ml, of thiosulphate solution used;

V_2 is the volume, in ml, of thiosulphate solution in the blank titrations;

T is the concentration, in mol/l, of the thiosulphate solution used;

m is the mass, in g, of the test sample;

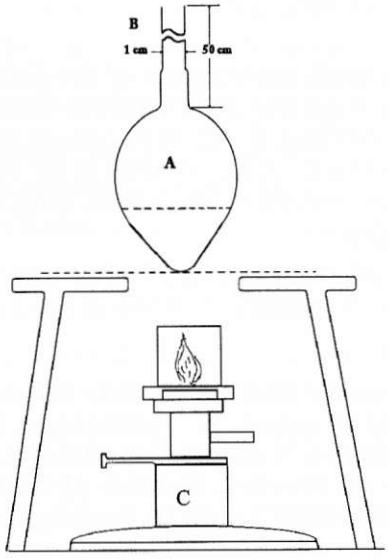
H is the moisture content, in g per 100 g of the sample.

9. References

9.1 RB Player and R Wood, J. Assoc. Publ. Analysts, 1980, 18, 29-40.

9.2 Ministry of Agriculture, Fisheries and Food, Food Safety Directorate, MAFF Validated Methods for the Analysis of Food, Introduction, General Considerations and Analytical Quality Control, J. Assoc. Publ. Analysts, 1992, **28**, 11-16.

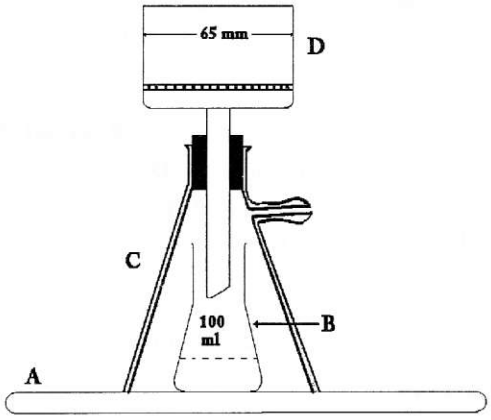
Fig. 1



Digestion assembly:

A, acetylation flask; B, air condenser; C, Argand burner.

Fig. 2



Filter flask arrangement:

A, glass plate; B, receiving flask; C, suction flask; D, Buchner funnel.

APPENDIX 1

Analytical Quality Control

General principles of analytical quality control are outlined in protocol V.0 of the series[2].

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 below (Table 1). At levels lower than 0.5 g of crude fibre per 100 g of dry sample, r may be taken as 0.05 g per 100 g; at higher levels, r would be expected to increase to over 0.2 g per 100 g and may be taken to be 10% of the observed level. This would correspond to a relative standard deviation of repeatability (coefficient of variance of repeatability), RSD_r, of 4% at the higher levels.

A2 Reproducibility

The absolute difference between two test results obtained under reproducibility conditions should not be greater than the reproducibility, R, deduced from the collaborative trial data summarised below (Table 1). At levels lower than 0.7 g of crude fibre per 100 g of dry sample, R may be taken as 0.25 g per 100 g; at higher levels, R would be expected to increase to over 0.6 g per 100 g and may be taken to be 36% of the observed level. This would correspond to a relative standard deviation of reproducibility (coefficient of variance of reproducibility), RSD_R, of 13% at the higher level.

A3 Trueness (Bias)

The collaborative trial established satisfactory precision parameters for the method; a partial check on the precision is possible as Samples C and D were prepared from fixed mixtures of the two single flours represented by Samples A and B. When the fibre contents of the former are calculated from the observed fibre contents of the latter, and expressed in g of crude fibre per 100 g of dry sample, the following comparisons can be made:

Sample C: calculated, 1.69; observed, 1.69

Sample D: calculated, 1.48; observed, 1.41.

The agreement is good, and the difference between the calculated and observed values for sample D is no higher than expected from the observed precision.

The accuracy of the method was not tested by spiking with known amounts of fibre. However, there is no reason to suspect systematic bias.

A4 Limit of Detection

This limit has not been established, but the collaborative trial data suggests an accuracy which, if maintained, corresponds to an extrapolated lower limit of roughly 0.05 g of crude fibre per 100 g of dry sample, for a single determination.

A5 Statistical Data Derived from the Results of Interlaboratory Tests

Participants in the collaborative trial each received four samples on which he was required to carry out duplicate determinations of:

moisture content, by drying at 103 ± 2°C to constant weight;

crude fibre content, by the method above (V26);

crude fibre content, by two other methods, which are not reported here.

The composition of the samples were:

A: 100% wholewheat stone-ground flour;

B: plain white flour;

C: a homogenised mixture of A and B in the ratio of 7:2,

D: a homogenised mixture of A and B in the ratio of 2:1.

Table 1 summarises the statistical data; the levels of analyte are expressed as g of crude fibre per 100 g of sample on a dry weight basis.

TABLE 1

Statistical Analysis of Crude Fibre (g/100g) in Flour

Sample	A	B	C	D
Number of Laboratories retained after eliminating outliers	16	16	16	16
Number of Laboratories eliminated as outliers	2	2	2	2
Number of results accepted after eliminating outliers	30	32	32	32
LEVEL OF ANALYTE				
Mean observed value $\bar{x}$	2.10	0.23	1.69	1.41
REPEATABILITY				
Standard Deviation S_r	0.072	0.016	0.094	0.068
Relative Standard Deviation RSD_r(%)	3.4	7.1	5.6	4.8
Repeatability r [2.8 × S_r]	0.202	0.046	0.264	0.190
REPRODUCIBILITY				
Standard Deviation S_R	0.236	0.082	0.213	0.148
Relative Standard Deviation RSD_R(%)	11	36	13	11
Reproducibility R [2.8 × S_R]	0.662	0.231	0.597	0.415

A6 Key to Table 1

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