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# Determination of Dietary Fibre in Cereals and Cereal Products—Collaborative Trials

## Part II: Study of a Modified Englyst Procedure

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In the light of the results of a previous trial to find a suitable method for measuring dietary fibre in cereal foods (Part I), a modified Englyst procedure for measurement of non-starch polysaccharides has been tested. Seventeen laboratories participated in this phase of the study by analysing blind duplicate samples of cereal foods including wholemeal, white, rye and pea-hull bread, high and low fibre brown bread, and a mixture of breads. In the trial, the method showed good repeatability within laboratories but the reproducibility between laboratories was unsatisfactory. Laboratories with experience of this type of analysis did significantly better than those without, possibly because of the lack of suitable equipment for carbohydrate chemistry in the latter. Other methods tested by some laboratories on the same samples were shown to be inaccurate for the measurement of NSP. As a result of this trial, it is recommended that the Englyst method be further modified to avoid the need for specialized equipment and expert knowledge of carbohydrate chemistry, and then be subjected to a new trial.

By the mid 1970s it was clear that dietary fibre consisted of a complex mixture of polysaccharides with different chemical and physiological properties. Although a number of methods for measurement of fibre had been developed, none was sufficiently accurate for food labelling or detailed enough to provide the necessary information for the investigation of the physiological properties of these dietary polysaccharides. The variable and often conflicting results reported in clinical experiments and epidemiological studies with fibre have highlighted the need for an accurate and more informative method for its measurement. It was therefore decided at the outset of the trials currently being reported[1] to determine dietary fibre as the sum of its chemical components. Lignin, which is virtually absent from human foods, was excluded from the method because it is not a carbohydrate, and for other reasons that were given in the Report on Part I of the trials[1]. Dietary fibre is defined, for analytical purposes, as the sum of the non-starch polysaccharides (NSP) and measured as the monosaccharides released by hydrolysis of NSP.

We have reported[1] the results of an inter-laboratory collaborative trial of 5 methods for the determination of dietary fibre in cereal foods. The study showed

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that widely differing values could be obtained for the same sample with different methods, and that considerable interlaboratory variation occurred with any given sample. No one method was satisfactory but it was felt by the Analytical Advisory Panel[1] that the Englyst procedure[2], used by 7 of the 22 participants, was the most accurate, gave the most useful information and offered the greatest potential for development as a statutory method.

A detailed description of the development of the Englyst procedure, and its validation is given elsewhere[2,3,4]. The aim of the procedure is to measure the non-starch polysaccharides in plant foods. In developing it, therefore, the main purpose has been to identify and completely recover this fraction without contamination from other polysaccharides such as starch. In the method, starch is gelatinised and then removed by enzymic digestion. The remaining polysaccharides are hydrolysed with 12 mol/l sulphuric acid and individual sugars measured by gas liquid chromatography as alditol acetate derivatives. Uronic acids are measured by a colorimetric method[5]. In addition to values for total NSP, small modifications allow values to be obtained for cellulose, and soluble and insoluble NSP.

**Removal of Starch**

A major problem in all methods for measurement of dietary fibre (NSP) is the removal of starch. In the present method, which depends on identification of component sugars after hydrolysis, starch must be removed completely so that any glucose present can be considered as deriving from NSP. Contamination with 2–3 per cent. of residual starch will lead to a substantial overestimate of NSP in foods such as potatoes, white bread, rice and cornflakes, in which NSP is a relatively small proportion of the total polysaccharides.

In the method used in the previous trial[1], starch was dispersed in acetate buffer before enzymic hydrolysis with a mixture of $\alpha$-amylase and pullulanase. This procedure does not completely remove starch, leaving behind a fraction resistant to enzymic digestion (resistant starch, RS). To obtain a figure for NSP, therefore, the result had to be corrected for RS, which was measured separately after dispersion with potassium hydroxide. For the present trial, the method has been modified to include an initial treatment of the sample with dimethylsulfoxide (DMSO), which renders all starch susceptible to enzymic hydrolysis.

Dimethylsulfoxide is known to be an effective agent for disrupting intermolecular hydrogen bonds[6,7] and has been used in the measurement of starch[8]. In preliminary studies, we observed that incubation in 100 per cent. DMSO, but not 90 per cent., for 24 h at room temperature enabled the dispersion of all starch from white bread. However, these conditions failed to disperse completely the starch granules from wheat flour or to redisperse the resistant starch from cooked potatoes or dried peas. Starch in these products, and in high amylose corn starch, was successfully dispersed with 100 per cent. DMSO at 100°C for 1 h. Following this treatment, effective hydrolysis with $\alpha$-amylase and pullulanase was obtained by diluting DMSO 1 : 5 with 0·1 mol/l sodium acetate buffer at pH 5·2. This treatment with DMSO does not cause any loss of NSP[3].

Two systems that measure NSP have been described. In one, starch is dispersed with acetate buffer and in the other with DMSO. For the present

study, analysts were asked to measure NSP after complete starch removal using DMSO. Some analysts also chose to follow the procedure detailed in the previous trial[1] and measured NSP + RS. In addition, some laboratories reported results using the method of Asp *et al.*[9], and the recently developed AOAC method[10].

## Collaborative Trial Organisation, Samples and Results

LABORATORIES AND METHODS

Seventeen analysts participated in the study. The method used was the Englyst procedure as previously tested[1], but with the modifications described above.

The dry matter content of each sample was obtained by drying the homogenised sample to constant weight at 103 (±2)°C.

SAMPLES

There were 7 samples analysed by participants. They were circulated as "blind" duplicates—i.e. each analyst received 14 samples and was asked to analyse each one once only.

The samples were:

(a) wholemeal bread
(b) brown bread, low fibre
(c) pea-hull bread
(d) rye bread
(e) white bread
(f) brown bread, high fibre
(g) 1 : 1 mixture of (e) and (f)

Samples were all prepared commercially by baking special lots. Each bake was thoroughly ground and homogenised before being sent to participants. Samples were coloured during preparation so that each appeared uniform when finally received by the analyst.

Analysts were asked to analyse each sample once only and to report the single result as a percentage by weight of the sample on a dry matter basis.

## Statistical Analysis

The data obtained were analysed using Cochran's and Dixon's Tests for outlying results with the procedures given by the British Standards Institution[11]. Some outlying results were found and these were not included in the calculation of means ($\bar{x}$), repeatabilities ($r$) nor reproducibilities ($R$), as defined by the British Standards Institution.

## Results

The results obtained by the participating analysts using the prescribed method are given in Table I. Results that were obtained using other methods are given in Table II. As may be seen from Table I, the method proved to be adequately repeatable in any given laboratory but there was substantial variation between laboratories, giving high reproducibility values (3·7–8·6); the greatest variability

being seen with wholemeal bread ($R = 8{\cdot}6$) and the mixture of breads ($R = 7{\cdot}4$). The mixture gave results that were slightly higher at 5·7 per cent. than might have been expected, as the sample was prepared from the white bread (3·3) and brown bread (6·75) samples used in the trial. Mean values for the whole group for each sample tended to be slightly higher than found by the Cambridge laboratory (laboratory 8).

The majority of experienced analysts commented that the method worked well and did not cause any major difficulties. A number of minor problems were encountered however in laboratories with less experience in carbohydrate analysis. These problems included the inadequate mixing of enzyme and sample during incubation, difficulties with the evaporation step and with the GLC determination of constituent sugars. More consistent difficulties were reported with the barium carbonate neutralisation stage, which several laboratories found slow, tedious and unsatisfactory. Some laboratories lacked equipment such as suitable glass tubes, a rotating device for incubation with enzyme and a vortex evaporator. This led to samples being left for prolonged periods during the method at times when this was not appropriate. Much of the inter-laboratory variation can probably be explained by the relative experience or inexperience of each collaborating team with this type of analysis. Laboratories with experience of, and equipment available for, similar carbohydrate chemistry showed good agreement but inexperienced and less well equipped laboratories did not.

Another reason for the discrepancies in the results relates to the use of GLC column packing material of doubtful quality. At the time of the trial, packing material bearing the label Supelcoport 100/200 mesh 3 per cent. SP 2320 (as recommended in the procedure) was produced by a firm other than Supelco Inc., Pennsylvania, U.S.A. This material was subsequently shown to be virtually useless for sugar analysis and this caused considerable problems for some participants.

In addition to the main trial procedure, where starch is dispersed by DMSO, six laboratories carried out the Englyst procedure using acetate buffer[2]. In contrast to DMSO, acetate does not disperse all starch, and RS is included in the value for total NSP. The results presented in Table II show (except for laboratory 15) good agreement within and between laboratories using this method for measuring NSP + RS. The good agreement between laboratories with this method resulted mainly because it was carried out by the more experienced analysts. Since RS is included in the measurement of NSP by this technique, the results in Table II are significantly higher than those obtained by dispersing starch with DMSO.

One experienced laboratory carried out measurements using the method of Asp *et al.*[9] and the AOAC procedure[10]. Results obtained by these procedures were in close agreement with each other (Table II) but even higher than the Englyst NSP + RS values. Both methods are based on gravimetric analysis of dietary fibre and share a number of features in common. Part of the difference between Englyst NSP + RS and these two methods can be attributed to "substances measuring as lignin", but this cannot be the explanation for food such as white bread. After completion of the trial, an interlaboratory study

TABLE I

DIETARY FIBRE COLLABORATIVE TRIAL RESULTS
PERCENTAGE OF DIETARY FIBRE BY SIMPLIFIED DUNN NSP METHOD. (DRY MATTER BASIS)

| | Sample type | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Wholemeal bread | | Brown bread low fibre | | Peahull bread | | Rye bread | | White bread | | Brown bread high fibre | | Mixture | |
| | Sample code numbers | | | | | | | | | | | | | |
| Laboratory | 2 | 10 | 3 | 14 | 5 | 11 | 8 | 12 | 1 | 7 | 4 | 6 | 9 | 13 |
| 1 | 7·31 | 7·13 | 3·95 | 3·35 | 4·64 | 5·19 | 1·89 | 2·12 | 1·19 | 1·05 | 3·91 | 3·88 | 3·52 | 3·05 |
| 2 | 5·68 | 5·84 | 5·43 | 5·27 | 5·01 | 7·33 | 3·30 | 3·44 | 8·73[a] | 5·69[a] | 4·78 | 7·32 | 4·55 | 4·38 |
| 3 | 9·45 | 9·26 | 5·33 | 5·52 | 6·01 | 7·30 | 3·22 | 3·28 | 2·71 | 2·59 | 6·23 | 7·44 | 4·69 | 4·38 |
| 4 | 8·74 | 7·79 | 0·20[a] | 10·27[a] | 0·72[a] | 15·02[a] | 3·05[a] | 5·64[a] | 8·20 | 5·89 | 5·38[a] | 0·09[a] | 10·02 | 10·69 |
| 5 | 10·59 | 11·08 | 6·30 | 6·13 | 6·92 | 7·50 | 4·12 | 3·81 | 2·88 | 2·81 | 8·94 | 9·11 | 5·61 | 5·89 |
| 6 | 10·28 | 10·72 | 7·19 | 5·88 | 6·38 | 7·66 | 5·07 | 5·51 | 3·31 | 3·19 | 7·38 | 9·10 | 5·12 | 5·94 |
| 7 | 11·47 | 11·74 | 6·50 | 7·12 | 8·75 | 8·29 | 5·42 | 5·99 | 4·37 | 4·52 | 8·04 | 8·23 | 7·66[b] | 6·77[b] |
| 8 | 10·70 | 10·28 | 6·37 | 5·86 | 7·67 | 7·60 | 3·81 | 3·81 | 3·25 | 3·13 | 7·37 | 7·69 | 5·56 | 5·26 |
| 9 | 12·34 | 13·45 | 9·05 | 10·06 | 10·64 | 10·28 | 6·59[a] | 7·70[a] | 8·10 | 7·69 | 12·80[b] | 12·78[b] | 9·08 | 9·45 |
| 10 | 5·30[a] | 12·09[a] | 5·89 | 6·39 | 8·79 | 7·34 | 4·25 | 4·05 | 2·63 | 3·80 | 7·49 | 8·58 | 5·84 | 6·14 |
| 11 | 4·89 | 6·92 | 3·44 | 4·08 | 4·71 | 4·60 | 2·59 | 2·41 | 1·61 | 2·65 | 4·36 | 6·29 | 3·29 | 3·88 |
| 12 | 10·22 | 12·12 | 6·01 | 5·79 | 7·06 | 7·68 | 2·78 | 2·86 | 2·38 | 2·21 | 8·74 | 7·24 | 4·84 | 4·40 |
| 13 | 11·11 | 11·11 | 6·52 | 6·25 | 7·42 | 7·79 | 3·95 | 3·38 | 3·84 | 3·67 | 7·99 | 8·05 | 5·32 | 5·96 |
| 14 | 10·95 | 12·12 | 6·34 | 6·33 | 8·25 | 7·16 | 5·37[a] | 8·13[a] | 3·16 | 2·72 | 7·20 | 7·83 | 5·24 | 4·71 |
| 15 | 14·34 | 14·28 | 4·60[a] | 10·94[a] | 5·54[a] | 14·94[a] | 4·43[a] | 11·93[a] | 2·62[a] | 7·91[a] | 6·97 | 6·29 | 10·58 | 12·19 |
| 16 | 2·39 | 2·30 | 1·12 | 1·27 | 1·70 | 1·81 | 0·68 | 0·70 | 0·59 | 0·51 | 1·73 | 1·55 | 1·09 | 1·30 |
| 17 | 8·93 | 9·17 | 5·24 | 5·62 | 6·99 | 7·60 | 3·97 | 3·82 | 2·64 | 2·79 | 6·51 | 6·35 | 5·22 | 5·03 |
| Mean $\bar{x}$ | 9·52 | | 5·65 | | 6·87 | | 3·47 | | 3·3 | | 6·75 | | 5·69 | |
| Repeatability $r$ | 1·74 | | 1·13 | | 1·92 | | 0·59 | | 1·49 | | 2·26 | | 1·20 | |
| Reproducibility $R$ | 8·65 | | 5·23 | | 5·76 | | 3·73 | | 5·50 | | 5·69 | | 7·44 | |

*Notes*

a. Result discarded in Cochran's test at $P \leqslant 0{\cdot}05$ level. Not included in the calculation of mean, repeatability and reproducibility.

b. Result discarded in Dixon's test at $P \leqslant 0{\cdot}05$ level. Not included in the calculation of mean, repeatability and reproducibility.

TABLE II

DIETARY FIBRE COLLABORATIVE TRIAL RESULTS
PERCENTAGE OF DIETARY FIBRE BY ALTERNATIVE METHODS FOR NSP. (DRY MATTER BASIS)

| | | Sample type | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Wholemeal bread | | Brown bread low fibre | | Peahull bread | | Rye bread | | White bread | | Brown bread high fibre | | Mixture | |
| | | Sample code numbers | | | | | | | | | | | | | |
| Laboratory | Method | 2 | 10 | 3 | 14 | 5 | 11 | 8 | 12 | 1 | 7 | 4 | 6 | 9 | 13 |
| 3 | NSP + RS[a] | 10·65 | 10·85 | 6·19 | 6·11 | 7·88 | 7·43 | 4·97 | 5·01 | 3·74 | 3·70 | 7·77 | 7·57 | 5·58 | 5·71 |
| 6 | NSP + RS | 10·63 | 10·44 | 7·12 | 6·46 | 7·22 | 8·57 | 5·83 | 5·78 | 5·10 | 4·12 | 8·08 | 8·28 | 7·32 | 6·56 |
| 8 | NSP + RS | 11·46 | 11·76 | 6·87 | 6·86 | 8·89 | 8·28 | 5·32 | 5·28 | 4·33 | 4·08 | 8·06 | 8·62 | 6·05 | 6·03 |
| 12 | NSP + RS | 11·85 | 12·20 | 6·52 | 6·99 | 8·39 | 8·10 | 4·53 | 4·90 | 3·77 | 3·51 | 8·27 | 8·49 | 7·65 | 5·99 |
| 13 | NSP + RS | 11·07 | 9·89 | 7·49 | 5·58 | 7·53 | 7·30 | 4·22 | 3·19 | 2·19 | 3·17 | 10·34 | 7·07 | 4·86 | 5·14 |
| 15 | NSP + RS | 10·65 | 20·06 | 5·16 | 11·56 | 6·72 | 19·23 | 4·93 | 12·11 | 2·87 | 2·71 | 6·94 | 6·83 | 5·04 | 12·60 |
| other[d] | Asp[b] | 13·6 | 13·2 | 7·7 | 8·2 | 8·9 | 8·9 | 5·5 | 6·1 | 4·8 | 4·5 | 9·7 | 9·9 | 7·0 | 7·2 |
| other[d] | AOAC[c] | 13·3 | 13·3 | 7·7 | 7·9 | 9·0 | 9·1 | 5·4 | 5·8 | 4·4 | 4·4 | 9·4 | 10·1 | 7·3 | 7·4 |

*Notes*

a. Englyst procedure, using acetate buffer for dispersing starch, Englyst *et al.*, 1982[2].
b. Asp *et al.,* 1983[9].
c. Prosky *et al.,* 1984[10].
d. Laboratory experienced with this method.

between the Department of Food Chemistry, Lund, Sweden and the Dunn Clinical Nutrition Centre, Cambridge was carried out in which it was shown that approximately half of the starch measured as RS by the Englyst procedure was removed in the procedure of Asp *et al.* Analysis of high and low-NSP cornflakes gave 2·8 g of RS/100 g for both samples by the Englyst procedure, but only 1·4 and 1·3 g of RS/100 g was recovered from the two samples by the Asp procedure. This remaining RS is included as dietary fibre in the Asp and AOAC procedures.

## Conclusion

The trial showed the Englyst method to be accurate and repeatable within laboratories, but the reproducibility between laboratories was not satisfactory. A major problem was the lack of suitable equipment and experience in this sort of chemistry. It is recommended that the method be further simplified and adapted to avoid the need for special equipment and then submitted to a new trial. Other methods tested in this trial gave values that were much greater than true NSP content of foods and the discrepancies cannot be accounted for on the basis of the presence of RS alone.

Following completion of this trial, the Englyst procedure has been further modified and subjected to validation by collaborative trial together with two potential quality control procedures. A report of that trial will appear in a subsequent issue of this Journal.

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In addition, other results were submitted by Laboratories not using the specified procedures.

## References

1. Cummings, J. H., Englyst, H. N., and Wood, R. J., *J. Assoc. Publ. Analysts*, 1985, **23**, 1–35.
2. Englyst, H., Wiggins, H. S., and Cummings, J. H., *Analyst,* 1982, **107**, 307.
3. Englyst, H. N., and Cummings, J. H., *Analyst,* 1984, **109**, 937–942.
4. Englyst, H. N., "Dietary Polysaccharide Breakdown in the Gut of Man". Ph.D. Thesis, University of Cambridge, 1985.
5. Scott, R. W., *Analyt. Chem.*, 1979, **51**, 936–941.

6. Erlander, S. R., and Tobin, T., *Makromol. Chem.*, 1967, **107**, 204.
7. Erlander, S. R., and Tobin, T., *Makromol. Chem.*, 1968, **111**, 194.
8. Libby, R. A., *Cereal Chem.*, 1970, **47**, 273.
9. Asp, N-G., Johansson, C-G., Hallmer, H., and Siljestrom, M., *J. Agric. Fd. Chem.*, 1983, **37**, 476–482.
10. Prosky, L., Asp, N-G., Furda, I., Devries, J. W., Schweizer, T. F., and Harland, B. F., *J. Assoc. Anal. Chem.*, 1984, **67**, 1044–1052.
11. British Standards Institution, "Precision of Test Methods". BS J497; Part 1. London, 1979.

## APPENDIX I

### MODIFIED ENGLYST PROCEDURE USED IN THE SECOND TRIAL

#### 1. SCOPE AND FIELD OF APPLICATION

The method determines the principal fraction of dietary fibre, the non-starch polysaccharides (NSP), in foods of cereal origin.

#### 2. DEFINITION

The content of dietary fibre: the content of non-starch polysaccharides 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. The resulting individual neutral sugars are determined by gas-liquid chromatography (GLC) of their alditol-acetate derivatives. Uronic acids are determined by a colorimetric procedure.

#### 4. REAGENTS

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

4.1 *Acetone*

4.2 *Dimethyl Sulphoxide*

4.3 *Sodium Acetate Buffer, 0·1 mol/l, pH 5·2:* Dissolve 13·608 g of sodium acetate trihydrate, $CH_3COONa.3H_2O$, in water saturated with benzoic acid, and make up to 1 litre with benzoic acid saturated water. Adjust to pH 5·2 with acetic acid 0·1 mol/l.

To stabilize and activate enzymes add 4 ml of calcium chloride solution, 1 mol/l, to 1 litre of buffer.

4.4 *α-Amylase Enzyme*

Hog pancreas α-amylase, EC 3.2.1.1 (Sigma A 4268) (specific activity is 20 units per mg. 1 ml of enzyme preparation contains 5 mg).

4.5 *Pullulanase Enzyme*

Pullulanase, EC 3.2.1.4.1 (Boehringer 108944) (100 units per ml).

4.6 *Mixed Enzyme Solution:* Containing 4000 units of α-amylase (4.4) and 5 units of pullulanase (4.5) per ml of sodium acetate buffer (4.3). Prepare fresh immediately before use.

4.7 *Ethanol, absolute*

4.8 *Ethanol, 85 per cent. v/v*

4.9 *Sulphuric Acid, 12 mol/l:* Standardised solution.

4.10 *GLC Internal Standard Solution*

Add 1 g of erythritol and 1 g of myo-inositol to water saturated with benzoic acid and make up to 1 litre with benzoic acid saturated water.

4.11 *Octan-2 ol*

4.12 *Barium Carbonate, $Ba\ CO_3$, Solid:* Use as a fine powder.

4.13 *Ammonium Hydroxide/Sodium Borohydride Solution:* Solution of ammonium hydroxide (3 mol/l) containing 100 mg of sodium borohydride ($NaBH_4$) per ml (ammonium hydroxide reagent strength is not critical).

4.14 *Methanol*

4.15 *Acetic Acid, glacial*

4.16 *Acetic Anhydride*

4.17 *Sodium Chloride/Boric Acid Solution:* Add 2 g of sodium chloride, NaCl, and 3 g of boric acid, $H_3BO_3$, to 100 ml of water. Shake to dissolve.

4.18 *Sulphuric Acid, concentrated*

4.19 *Dimethylphenol Solution:* Add 0·1 g of 3,5-dimethylphenol ($(CH_3)_2$-$C_6H_3.OH$), to 100 ml of glacial acetic acid (4.15). Shake to dissolve.

4.20 *Glucuronic Acid:* Solid, for preparation of standards used in uronic acid determination.

4.21 *Standard Sugar Solutions*

Weigh 250 mg of each of the following sugars (dried to constant weight under vacuum with $P_2O_5$) into separate 500 ml volumetric flasks:

L—rhamnose
L—arabinose
D—xylose
D—mannose
D—galactose
D—glucose.

Add 250 mg of both erythritol and myo-inositol to each flask. Dissolve the contents in, and make up to volume with, water saturated with benzoic acid which has been diluted 1 : 5 before use.

Prepare a single standard solution containing 0·500 g/l of each of the above six standard sugars and 0·500 g/l of erythritol and 1·000 g/l of myo-inositol.

Reduce and acetylate 2 ml of each of the standard solutions as described below for the sample (see Sections 6.6.1.1 and 6.6.1.2).

## 5. APPARATUS

5.1 *Centrifuge:* Capable of centrifuging to at least 2500 r.p.m. (i.e. of the order of 1500 g).

5.2 *Centrifuge Tubes:* Glass centrifuge tubes of 50 ml capacity and fitted with screw-tops.

5.3 *Magnetic Stirrer Hot Plate:* Fitted with a beaker of water. The temperature is variously adjusted during the course of the procedure. If necessary, cover the beaker to maintain boiling.

5.4 *Magnetic Stirrers, PTFE-coated:* To fit the centrifuge tubes (5.2).

5.5 *Evaporator*

Buchler Vortex evaporator with capacity to take 56 × 15 ml tubes, or an equivalent evaporator.

5.6 *GLC Chromatograph*

GLC Chromatograph fitted with flame ionisation detector and, if possible, a peak area integrator.

5.7 *GLC Column and Conditions*

The GLC column and conditions must be selected to give separation of the alditol acetate derivatives of the individual sugars. The method has been developed on the basis of the following conditions, but others are available:

Glass column, 2·1 m × 2 mm i.d., packed with Supelcoport (100/120 mesh) coated with 3 per cent. SP 2330.
Injector temperature : 250°C
Column temperature : 215°C (isothermal)
Detector temperature: 250°C
Carrier gas : nitrogen
Carrier gas flow-rate: 25 ml/min.

Typical chromatograms are given in Appendix I. If the erythritol internal standard is not isolated or quantified, repack the first 5 cm of the GLC column and recondition.

## 6. PROCEDURE

6.1 *Pre-treatment of Samples*

As far as possible foods are analysed without any pre-treatment. If there are problems in taking a representative sample, the foods with a low water content are ball-milled for 2–3 min, and foods with a higher water content are homogenised, or freeze dried and ball-milled.

6.2 *Analysis Sample*

Accurately weigh, to the nearest 0·1 mg, between 200 and 500 mg of sample (but containing not more than 200 mg of dry matter), into a 50 ml screw-top centrifuge tube (5·2) and add a stirrer.

6.3 *Fat Extraction*

Dry samples (i.e. 90–100 per cent. dry matter) with less than 2–3 per cent. fat may be analysed directly. Otherwise, add 40 ml of acetone (4.1), mix for 30 minutes using a magnetic stirrer, centrifuge and remove by aspiration as much of the supernatant liquor as possible without disturbing the residue. Air-dry the residue or dry it by mixing at 62·5 ± 2.5°C (see 6.4.2).

6.4 *Dispersion and Enzymic Hydrolysis*

To the sample or residue, add 2 ml of dimethyl sulphoxide (4.2) and heat in a boiling water bath for 1 h, timed from when re-boiling commences, stirring continuously. Then without cooling, add 8 ml of sodium acetate buffer (4.3) at 50 ± 5°C, and immediately mix with a whirlimixer.

Cool the tube to 42 ± 2°C and immediately add 0·2 ml of mixed enzyme solution (4.6).

Incubate the sample at 42 ± 2°C for 16–18 h mixing continuously. After the enzyme treatment add 40 ml of absolute ethanol (4.7), mix well and leave to stand for at least 1 h at ambient temperature. Centrifuge at 2500 r.p.m. for 10 min or until a clear supernatant liquid is obtained. Remove by aspiration as much of the supernatant liquor as possible without disturbing the residue and discard it. Wash the residue twice with 50 ml of 85 per cent. ethanol (4.8) each time by mixing to form a suspension of the residue, centrifuging until clear and removing the supernatant liquor as previously.

Add to the washed residue 40 ml of acetone (4.1), stir for 5 min and then centrifuge at 2500 r.p.m. for approximately 10 min until clear. Remove the supernatant liquor by aspiration and discard it.

Place the tube in a water bath at 62·5 ± 2·5°C on the magnetic stirrer hot plate and mix the residue for a few minutes until it appears to be dry. The beaker may be covered and the acetone vapour removed by water pump. Care must be taken to avoid loss of material at this stage.

6.5 *Acid Hydrolysis of the Residue from Enzymic Digestion*

Disperse the dried residue from Section 6.4 in 2 ml of 12 mol/l sulphuric acid (4.9) using a whirlimixer and the stirrer. Leave at 35 ± 2°C for 1 to 1.25 h to solubilise the cellulose and then rapidly add 12 ml of water, followed by 10 ml of GLC internal standard solution (4.10).

Mix the contents of the centrifuge tube.

Place in a boiling water bath for 2 h timed from re-boiling, stirring continuously. Remove approximately 14 ml (for the determination of uronic acids—see 6.7). To the remainder (approximately 10 ml) add 1 drop of octan-2-ol (4.11), 1·5–2·0 g of barium carbonate (4.12). Mix with whirlimixer and then the magnetic stirrer at 40 ± 2°C. After the 10 min, add a further 1·5–

2·0 g of barium carbonate (4.12) and mix for 10–20 min at 40 ± 2°C as above until neutral (by pH paper).

Centrifuge and take 2 ml of the supernatant liquor for the determination of the neutral sugars by GLC.

## 6.6 *GLC Determination of Neutral Sugars*

### 6.6.1 *Preparation of Alditol Acetates*

#### 6.6.1.1 *Reduction*

To 2 ml of neutral supernatant liquor in Procedure 6.5, add 0·1 ml of ammonium hydroxide/sodium borohydride solution (4.13). Mix and leave for at least 2 h but preferably overnight at ambient temperature. Add 0·5 ml of methanol (4.14), 0·1 ml of glacial acetic acid (4.15) and evaporate to dryness at 40°C.

Add a further 3 ml of methanol (4.14) to the residue and evaporate to dryness. Repeat this last step twice.

Similar amounts (2 ml) of the standard solutions (4.21) are treated in the same way.

#### 6.6.1.2 *Acetylation*

To the dry residues from 6.6.1.1, including the sugar standards, add 0·3 ml of acetic anhydride (4.16), put on the screw-cap, mix well and heat for 2 h at 120°C with frequent shaking during the first 10 min.

### 6.6.2 *GLC Determination*

6.6.2.1 Carry out conventional GLC determination of the prepared alditol acetate derivatives of the neutral sugar solutions obtained from Section 6.6.1.2 using the standards prepared from the standard sugar solutions in Section 4.21, GLC columns and conditions given in Sections 5.6 and 5.7, and taking 1–2 μl of the supernatant solutions for injection.

## 6.7 *Uronic Acids*

Mix 0·3 ml of supernatant liquid obtained from Section 6.5 (diluted, if necessary, so that it contains between 25 and 100 μg/ml of uronic acids) with 0·3 ml of sodium chloride/boric acid mixture (4.17). Add 5 ml of concentrated sulphuric acid (4.18) and mix on a whirlimixer. Place the tube (40–50 ml) in a heating block set at 70°C. Leave for 40 ± 1 min and then cool to room temperature by placing in water.

When cool, add 0·2 ml of dimethylphenol solution (4.19) and mix immediately. Between 10 and 15 min later read the absorbance at 400 and 450 nm in a spectrophotometer against a water reference cell.

Subtract the reading at 400 nm from that at 450 nm for each sample. Plot the difference in absorbances obtained for glucuronic acid (4.20) standards (over the range 25–125 mg/ml) and read the sample concentrations from the graph.

## 7. EXPRESSION OF RESULTS

### 7.1 *Calculation of Neutral Sugars*

Calculate the amount of each sugar in g/100 g by the following formula:

$$\frac{\text{mg of Internal Standard} \times \text{Response for Sugar} \times 100}{\text{Response for Internal Standard} \times \text{mg of Sample}}$$

Use myo-inositol as the internal standard.

### 7.2 *Corrections*

Experiments with pure sugar mixtures and with polysaccharides have shown that the hydrolysis and derivatisation procedures result in losses of approximately 10 per cent. of sugars. In order to express NSP as polymers or anhydro sugars the GLC results should be decreased by approximately 10 per cent. Because these two corrections are approximately of the same value the GLC results are calculated and then directly expressed as polymers or anhydro sugars.

There is incomplete hydrolysis and acetylation of any rhamnose in the samples. This is corrected by applying a ×2 factor to the rhamnose value as determined experimentally. In practice, only traces of rhamnose are present in cereal products.

### 7.3 *Total Non-Starch Polysaccharides*

NSP = Arabinose + Xylose + Galactose + Glucose + Mannose + Uronic Acids + 2 × Rhamnose.

NSP is then expressed as polymers in g/100 g of material taken for analysis.

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# Determination of Dietary Fibre in Cereals and Cereal Products—Collaborative Trials
# Part III: Study of Further Simplified Procedures

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A collaborative trial has been carried out in 19 laboratories to compare methods of analysis for dietary fibre, defined as total non-starch polysaccharides (NSP). The methods tested were the Englyst procedure with GLC end-point determination, the Englyst method with colorimetric end-point determination, and a rapid method, based on the pentose content of cereal products, developed by the Flour Milling and Baking Research Association (FMBRA). The last two techniques were designed to be quality control procedures. The results of all three methods are satisfactory when compared with those from trials already carried out using previously published procedures for dietary fibre. The Englyst procedures permit the measurement of total NSP together with resistant starch, as well as total NSP by itself.

The Englyst procedure with GLC end-point determination can be recommended for inclusion in legislation.

The need to establish a definition for dietary fibre and to agree on a method of analysis to measure it has been discussed in previous reports[1,2]; it was concluded that there is a need for an agreed method of analysis and that dietary fibre should be defined for analytical purposes as "non-starch polysaccharides (NSP)"[1]. In addition, the draft Guidelines on Nutrition Labelling recently issued by Ministry of Agriculture, Fisheries and Food (MAFF) stipulate that a definition and method of analysis need to be developed and agreed[3].

In an attempt to agree such a method, MAFF has sponsored collaborative trials to evaluate methods for the determination of dietary fibre, and the results have been published[1,2].

In the first trial, a number of different techniques for the estimation of "dietary fibre" were assessed[1], from which it was concluded that the Englyst method was the most accurate and gave the most information about dietary fibre in a food. In the second trial, a modified Englyst procedure was used[2], and shown to be repeatable within laboratories but the reproducibility was unsatisfactory between laboratories. It was noted that significantly better results were obtained where there was experience of this type of analysis, largely because of the availability of suitable equipment. Laboratories unfamiliar with carbohydrate chemistry techniques produced less consistent results.

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On completion of the second trial, it was decided to simplify the Englyst procedure further in order to reduce the need for special equipment, whilst retaining the capacity of the method to provide detailed information about NSP from an analysis of its constituent sugars using a gas-liquid chromatograph (GLC). This modified method has now been subjected to a collaborative trial and the results are given in this Report.

In addition, two methods suitable for use as quality control procedures have been tested. One of these methods is the Englyst procedure further simplified by incorporating measurement by colorimetry of the constituent sugars released from NSP, thus obviating the need for a GLC; the other method is a procedure developed at the Flour Milling and Baking Research Association (FMBRA), Chorleywood, based on the measurement of pentose as a marker for total NSP. It is described in detail elsewhere[4].

**Definition of Dietary Fibre and Interference from Starch**

Early in the development of the dietary fibre hypothesis it was apparent that fibre could not be defined for human nutrition as crude fibre, since crude fibre bears little relation to the plant cell wall polysaccharides that are associated with physiological changes in man. In 1972, Trowell redefined fibre as "the skeletal remains of plant cells that are resistant to digestion by enzymes of man"[5]. With the development of interest in dietary fibre has come the need for an accurate method for its measurement. However, analysts have found it difficult to establish which part of plant material should be measured as dietary fibre. For analytical purposes, a precise chemical objective is needed. In the light of subsequent research, Trowell in 1985[6] concluded that "At the present time there is considerable international agreement concerning the principal constituents of dietary fibre. They are all polysaccharides, mainly cellulose, hemicellulose and pectic substances, conveniently designated non-starch polysaccharides (NSP)". This gives the analyst a clear task; to identify and measure the chemically well-defined non-starch polysaccharides.

Dietary fibre defined as NSP consists of plant polysaccharides sharing the common feature that they do not contain α-glucosidic linkages. Dietary fibre defined in this way includes the cell-wall and related plant polysaccharides, but does not include starch (α-glucans) in any form.

With the introduction of gas-liquid chromatography for measurement of the constituent sugars of NSP it became apparent that starch was interfering in the measurement of dietary fibre[7]. In starch-rich foods some starch was not being removed, due partly to insufficient gelatinisation and partly to the use of less potent enzymes for starch hydrolysis. A small amount of the starch in processed foods was shown to resist dispersion in boiling water and hydrolysis with pancreatic amylase[7]. If not removed, this starch is included in the measurement of NSP, leading to an unrealistically high proportion of NSP-glucose. Subsequently, it was shown that the starch resisting hydrolysis could be dispersed with potassium hydroxide and then hydrolysed with amyloglucosidase. This particular fraction of starch was termed resistant starch (RS), and defined as starch resistant to dispersion in boiling water and hydrolysis with

pancreatic amylase and pullulanase[7]. An accurate measurement of NSP in foods can be obtained only when RS is taken into account.

The amount of RS formed during the processing of starchy foodstuffs is influenced by many factors, including water content, pH, heating temperature and time, number of heating/cooling cycles, freezing, drying and the ratio of amylose to amylopectin[8]. The RS content of starchy food therefore depends to a large extent on the type of processing the food has undergone. The RS content

TABLE I

DIETARY FIBRE COLLABORATIVE TRIAL RESULTS FOR WHOLEMEAL BREAD BY THE ENGLYST GLC END-POINT METHOD
(Pre-Trial Sample—identical with Sample 10/11)

| | *g/100 g dry matter* | | | | | |
|---|---|---|---|---|---|---|
| Laboratory | Non starch polysaccharides (NSP) | | NSP + resistant starch (RS) | | Resistant starch | |
| 1 | 10·63, | 10·41 | 11·53, | 11·45 | 0·87, | 0·93 |
| 2 | 10·56, | 10·78 | 10·68, | 12·25 | 0·64, | 0·92 |
| 3 | 8·47, | 8·71 | 10·11, | 9·93 | 1·07, | 1·12 |
| 4 | 10·55, | 10·30 | 11·64, | 11·70 | 1·16, | 1·11 |
| 5 | 11·4, | 10·8 | 11·6, | 11·3 | −0·3[e] | −0·2[e] |
| 6 | 7·10, | 7·68 | 9·12, | 8·91 | 1·34, | 0·97 |
| 7 | 10·96, | 10·35 | 11·32, | 12·09 | 0·36[c], | 1·74[c] |
| 8 | 8·81, | 9·08 | 10·02, | 9·64 | 1·21, | 0·56 |
| 9 | 11·03, | 11·34 | 11·51, | 11·50 | 0·84, | 0·71 |
| 10 | 8·87, | 10·54 | 8·92, | 10·72 | 0·43, | 0·44 |
| 11 | 9·36, | 8·16 | 10·23, | 9·48 | 0·87, | 1·34 |
| 12 | 9·8, | 9·2 | 10·4, | 10·0 | 0·6, | 0·6 |
| 13 | 9·44, | 9·52 | 9·97, | 10·11 | 1·41, | 0·88 |
| 14 | 9·95, | 9·36 | 9·85, | 10·50 | 0·66, | 0·95 |
| 15 | 9·66, | 10·63 | 12·24, | 11·56 | 1·34, | 1·04 |
| 16 | 7·2, | 9·4 | 8·6, | 7·7 | 0·9, | 0 |
| 17 | 7·24, | 8·01 | 9·17, | 9·99 | 1·27, | 1·13 |
| 18 | —[a] | | —[a] | | —[a] | |
| 19[b] | — | | 9·9 | | — | |
| Mean ($\bar{x}$) | 9·57 | | 10·46 | | 0·85 | |
| Repeatability ($r$) | 1·73 | | 1·51 | | 0·74 | |
| Reproducibility ($R$) | 3·50 | | 3·27 | | 1·10 | |

For key, see Table XX.

of a number of widely available cereal foods has been measured[9]. The actual amount of RS is small, being less than 1 per cent. in bread. The highest content of RS, 3·1 per cent., is found in cornflakes. On the whole, these RS values are nutritionally insignificant. The interest in RS in starchy foods lies in the fact that it is technically possible to increase these values to 20 per cent. or more and, consequently, to make a substantial difference to the site of digestion and physiological properties of these foods in man.

Using ileostomy subjects, a series of test meals have been fed to permit observation of the digestion of various types of starch and NSP in the small intestine of man[10–12]. These studies have clearly shown that although RS largely

escapes digestion in the small intestine it is only a small proportion of the substantial amount of starch to do so. Starch escaping digestion in the small intestine cannot therefore be quantified or described by measuring only RS in food. This has led to investigation of the chemical and physical structure of starch, and to a new classification of starch based on its digestibility[8]. Starch escaping digestion in the small intestine may well be an important food

TABLE II

DIETARY FIBRE COLLABORATIVE TRIAL RESULTS FOR WHOLEMEAL BREAD BY ENGLYST COLORIMETRIC END-POINT METHOD

(Pre-Trial Sample—identical with Sample 10/11)

| | *g/100 g dry matter* | | | | | |
|---|---|---|---|---|---|---|
| Laboratory | Non starch polysaccharides (NSP) | | NSP + resistant starch (RS) | | Resistant starch | |
| 1 | 11·51, | 11·42 | 12·15, | 12·26 | 0·64, | 0·84 |
| 2 | 11·14, | 11·23 | 12·16, | 12·16 | 1·02, | 0·93 |
| 3 | 10·13, | 10·44 | 11·52, | 11·85 | 1·41, | 1·41 |
| 4 | 10·44, | 9·97 | 10·92, | 10·60 | 0·48, | 0·63 |
| 5 | 11·4, | 11·3 | 13·0, | 12·8 | 1·6, | 1·5 |
| 6 | 9·01, | 9.68 | 9·92, | 10·76 | 0·91, | 1·08 |
| 7 | 12·95, | 12·25 | 13·46, | 13·44 | 0·51, | 1·19 |
| 8 | 10·49, | 10·93 | 11·54, | 10·83 | 1·05 | −0·10[e] |
| 9 | 10·90, | 11·27 | 11·55, | 11·55 | 0·65, | 0·28 |
| 10 | 11·6, | 11·5 | 12·0, | 11·9 | 0·4, | 0·4 |
| 11 | 11·53, | 10·39 | 12·13, | 11·16 | 0·6, | 0·77 |
| 12 | 8·6, | 8·8 | 9·8, | 10·5 | 1·2, | 1·7 |
| 13 | 9·71, | 8·40 | 11·69, | 10·31 | 1·98, | 1·91 |
| 14 | 11·23, | 11·04 | 11·73, | 11·69 | 0·50, | 0·65 |
| 15 | 10·04, | 11·27 | 10·95, | 11·95 | 0·91, | 0·68 |
| 16 | 9·7, | 10·6 | 10·6, | 10·5 | 0·9, | 0·0 |
| 17 | 9·46, | 9·67 | 10·70, | 10·19 | 1·24, | 0·52 |
| 18 | —[a] | | —[a] | | —[a] | |
| 19[b] | 8·0, | 5·0 | 13·0, | 14·0 | 10·0, | 4·0 |
| Mean ($\bar{x}$) | 10·59 | | 11·48 | | 0·90 | |
| Repeatability ($r$) | 1·29 | | 1·20 | | 0·91 | |
| Reproducibility ($R$) | 3·00 | | 2·69 | | 1·42 | |

For key, see Table XX.

component in its own right. If it proves to be so, a case will need to be made for the measurement of all the dietary starch that escapes digestion, and not just the currently measured RS.

There are marked differences between starch and NSP, both analytically and in their role in the small and large intestine. It is therefore essential that NSP and starch escaping digestion in the small intestine are measured separately.

## Lignin

Lignin has not been included in the analytical definition of dietary fibre. Lignin is not a carbohydrate and should not be grouped analytically with NSP. It is a minor component of the human diet and, with the exception of cereal bran,

few foods contain measurable quantities. Lignin is difficult to determine chemically[13], and none of the present dietary fibre methods that include a value for it can justify this on strict chemical grounds. These methods simply isolate a collection of material (including Maillard and other food degradation products) which is better referred to as "substances measuring as lignin". Inclusion in the measurement of dietary fibre of an imprecise lignin value with an accurate

TABLE III

DIETARY FIBRE COLLABORATIVE TRIAL RESULTS FOR WHOLEMEAL BREAD BY ENGLYST GLC END-POINT METHOD
(Sample Code 10/11—identical with pre-trial sample)

| | *g/100 g dry matter* | | | | | |
|---|---|---|---|---|---|---|
| Laboratory | Non starch polysaccharides (NSP) | | NSP + resistant starch (RS) | | Resistant starch | |
| 1 | 10·35, | 10·03 | 10·98, | 10·75 | 0·79, | 0·87 |
| 2 | 9·00, | 9·15 | 10·02, | 10·04 | 1·0, | 0·90 |
| 3 | 12·03, | 11·97 | 13·31, | 13·36 | 1·04, | 1·13 |
| 4 | 10·28, | 9·66 | 11·51, | 11·32 | 0·98, | 0·94 |
| 5 | 11·05, | 11·46 | 14·46, | 12·53 | 1·81, | 0·82 |
| 6 | 10·04, | 8·15 | 9·00, | 9·86 | 0·0, | 1·01 |
| 7 | 10·28, | 10·76 | 12·27, | 11·60 | 1·42, | 1·05 |
| 8 | 7·76, | 8·30 | 8·74, | 8·90 | 0·92, | 0·74 |
| 9 | 9·36, | 9·69 | 10·02, | 10·30 | 0·82, | 0·48 |
| 10 | 7·62, | 8·10 | 8·72, | 9·14 | 0·83, | 0·85 |
| 11 | 7·18, | 7·14 | 9·30, | 7·63 | 1·18, | 0·58 |
| 12 | 9·6, | 11·3 | 13·6, | 15·5 | 1·8, | 1·3 |
| 13 | 8·65, | 10·29 | 12·80, | 11·05 | 1·09, | 0·76 |
| 14 | 8·76, | 8·72 | 11·81, | 11·74 | 2·48[d] | 2·22[d] |
| 15 | 7·84, | 8·11 | 9·15, | 9·25 | 0·89, | 0·90 |
| 16 | 8·4, | 9·6 | 8·8, | 9·4 | 0·5, | 0·4 |
| 17 | 9·45, | 10·23 | 10·10, | 10·88 | 0·65, | 0·76 |
| 18 | 7·8, | 7·9 | 7·95, | 9·01 | 0·6, | 1·0 |
| 19[b] | 6·0, | 8·0 | 9·7, | 8·0 | 2·7, | 0·8 |
| Mean ($\bar{x}$) | 9·33 | | 10·69 | | 0·91 | |
| Repeatability ($r$) | 1·69 | | 1·94 | | 0·87 | |
| Reproducibility ($R$) | 3·83 | | 5·40 | | 1·00 | |

For key, see Table XX.

measure of NSP is self-defeating; even if precise values for lignin were available, inclusion would invalidate the information obtained by measurement of NSP. If lignin is shown to be an important dietary constituent, then it should be measured separately; as should any other plant cell wall-associated substance, such as silica, cutin, etc.

## Methods Used in the Trial

1. ENGLYST PROCEDURE FOR THE DETERMINATION OF DIETARY FIBRE AS NSP: MEASUREMENT BY GAS LIQUID CHROMATOGRAPHY (GLC) OF CONSTITUENT SUGARS RELEASED FROM NSP

The previous trial showed that three steps in the procedure caused particular problems for some of the participants[3].

1. Adequate starch removal without having access to an effective, but nevertheless expensive, mixing device originally described for the method.
2. Neutralisation of hydrolysate with barium carbonate.
3. Repeated evaporation of batches of samples when preparing derivatives for GLC without access to an Evapomix.

These problems have been overcome by using pancreatin for starch hydrolysis instead of purified amylase, and by modifying the method of preparation of alditol acetates using *n*-methyl imidazole as catalyst. Full details of the new technique and its validation are given elsewhere[14,15]. The working procedures as used by the trial participants are given as Appendix I.

TABLE IV

DIETARY FIBRE COLLABORATIVE TRIAL RESULTS FOR WHOLEMEAL BREAD BY ENGLYST COLORIMETRIC METHOD

(Sample Code 10/11—identical with pre-trial sample)

| Laboratory | Non starch polysaccharides (NSP) g/100 g dry matter | | NSP + resistant starch (RS) | | Resistant starch | |
|---|---|---|---|---|---|---|
| 1 | 10·77, | 10·71 | 11·67, | 11·35 | 0·90, | 0·64 |
| 2 | 10·92, | 11·21 | 11·68, | 11·68 | 0·76, | 0·47 |
| 3 | 12·30, | 12·07 | 12·70, | 12·48 | 0·40, | 0·41 |
| 4 | 8·14, | 9·89 | 11·11, | 10·80 | 2·97, | 0·91 |
| 5 | 11·80, | 11·56 | 13·25, | 12·10 | 1·45, | 0·54 |
| 6 | 11·71, | 9·93 | 9·86, | 12·42 | −1·85[e], | 2·49 |
| 7 | 10·74, | 10·99 | 12·34, | 11·79 | 1·60, | 0·80 |
| 8 | 8·27, | 8·86 | 9·14, | 9·40 | 0·87, | 0·54 |
| 9 | 10·69, | 9·98 | 12·02, | 12·21 | 1·33, | 2·23 |
| 10 | 11·35, | 11·44 | 11·90, | 12·38 | 0·55, | 0·94 |
| 11 | 8·6, | 8·95 | 9·5, | 9·85 | 0·9, | 0·87 |
| 12 | 7·8, | 8·5 | 9·5, | 6·6 | 1·7, | 0·1 |
| 13 | 11·11, | 10·75 | 11·49, | 11·80 | 0·38, | 1·05 |
| 14 | 9·42, | 10·40 | 11·91, | 11·85 | 2·49, | 1·45 |
| 15 | 11·15, | 11·69 | 11·23, | 11·55 | 0·08, | −0·14[e] |
| 16 | 14·5, | 12·3 | 14·2, | 12·7 | 0·0, | 0·6 |
| 17 | 7·68, | 8·10 | 8·63, | 8·14 | 0·95, | 0·04 |
| 18 | 14·7, | 14·9 | 15·4, | 15·6 | 0·7, | 0·7 |
| 19[b] | 8·0, | 7·0 | 12·0, | 10·0 | 4·0, | 3·0 |
| Mean ($\bar{x}$) | 10·66 | | 11·45 | | 0·91 | |
| Repeatability ($r$) | 1·79 | | 2·11 | | 2·02 | |
| Reproducibility ($R$) | 5·23 | | 5·26 | | 2·09 | |

For key, see Table XX.

## 2. Englyst procedure for the determination of dietary fibre as NSP: measurement of constituent sugars by colorimetry

In this method, the fractionation technique from the Englyst procedure has been combined with a colorimetric method for measurement of reducing sugars[16]. This technique permits the determination of soluble and insoluble NSP, but does not characterise these fractions by measurement of individual

sugars. However, such detailed information is not required for most routine analyses. The colorimetric procedure is simple and rapid, and obviates the need for expensive GLC equipment. The colorimetric procedure described for the present trial has been compared with results obtained by GLC for a large number of cereals, vegetables and fruits. This and the validation of the procedure is reported elsewhere[17]. The working procedures as used by the trial participants are given as Appendix II.

TABLE V
DIETARY FIBRE COLLABORATIVE TRIAL RESULTS FOR WHITE BREAD, BY ENGLYST GLC END-POINT METHOD
(Sample Code 4/13)

| | *g/100 g dry matter* | | | | | |
|---|---|---|---|---|---|---|
| Laboratory | Non-starch polysaccharides (NSP) | | NSP + resistant starch (RS) | | Resistant starch | |
| 1 | 2·70, | 2·53 | 3·64, | 3·72 | 1·04, | 1·10 |
| 2 | 3·09, | 2·8 | 4·32, | 4·05 | 1·30, | 1·21 |
| 3 | 3·07, | 3·39 | 4·76, | 4·92 | 1·36, | 1·42 |
| 4 | 3·06, | 2·51 | 3·95, | 3·89 | 1·11, | 1·15 |
| 5 | 3·15, | 3·58 | 4·05, | 4·75 | 1·07, | 1·41 |
| 6 | 2·23, | 2·33 | 3·92, | 3·71 | 1·35, | 1·29 |
| 7 | 2·12, | 3·32 | 4·39, | 4·66 | 2·35[c], | 1·37[c] |
| 8 | 2·41, | 2·49 | 3·47, | 3·51 | 1·11, | 1·12 |
| 9 | 3·16, | 3·60 | 4·81, | 5·05 | 1·25, | 1·34 |
| 10 | 2·02, | 2·16 | 3·05, | 2·79 | 0·98, | 0·82 |
| 11 | 2·35, | 1·80 | 2·47, | 2·96 | 0·67, | 0·97 |
| 12 | 3·2, | 3·8 | 7·2[c], | 4·0[c] | 2·9[c], | 1·1[c] |
| 13 | 3·22, | 3·08 | 3·87, | 4·17 | 1·16, | 1·18 |
| 14 | 4·07, | 3·74 | 5·39, | 5·66 | 2·06[d], | 2·41[d] |
| 15 | 2·14, | 2·34 | 3·64, | 3·64 | 1·32, | 1·19 |
| 16 | 2·5, | 2·2 | 3·6, | 3·1 | 1·1, | 1·0 |
| 17 | 2·39, | 2·64 | 3·46, | 3·45 | 1·03, | 1·12 |
| 18 | 2·4, | 2·5 | 3·7, | 3·0 | 1·2, | 0·8 |
| 19[b] | 3·7[b], | –[b] | –[b], | [b] | –[b], | –[b], |
| Mean ($\bar{x}$) | 2·78 | | 3·93 | | 1·14 | |
| Repeatability ($r$) | 0·86 | | 0·68 | | 0·35 | |
| Reproducibility ($R$) | 1·63 | | 2·15 | | 0·51 | |

For key, see Table XX.

## 3. Rapid determination of dietary fibre in wheat products from measurement of pentose content (FMBRA method)

This procedure is based on previous detailed analyses using Southgate's and Englyst's procedures[18,7]. Such analyses show a relation between the NSP pentose content and total NSP in a cereal food (wheat). Using information about the monosaccharide composition of the NSP in a food, total NSP can be calculated from a measurement of pentose alone for wheat products.

A colorimetric method for rapid estimation of pentose in white flour has been adapted for use with products of a wide range of dietary fibre contents, by introducing a prehydrolysis step with 0·5 mol/l $H_2SO_4$ for 0·5 h. Pentose is

measured as xylose using a phloroglucinol reagent. Validation and application of this method has been described by the Flour Milling and Baking Research Association (FMBRA)[4]. The working procedures as used by the trial participants are given as Appendix III.

TABLE VI
DIETARY FIBRE COLLABORATIVE TRIAL RESULTS FOR WHITE BREAD, BY ENGLYST COLORIMETRIC END-POINT METHOD
(Sample Code 4/13)

| Laboratory | *g/100 g dry matter* Non-starch polysaccharides (NSP) | | NSP + resistant starch (RS) | | Resistant starch | |
|---|---|---|---|---|---|---|
| 1 | 2·98, | 3·11 | 4·15, | 4·18 | 1·17, | 1·07 |
| 2 | 3·50, | 3·64 | 4·66, | 4·98 | 1·16, | 1·34 |
| 3 | 3·46, | 3·58 | 5·12, | 5·37 | 1·66, | 1·79 |
| 4 | 5·27[c], | 1·90[c] | 4·78, | 4·82 | nil[c], | 2·92[c] |
| 5 | 3·43, | 3·97 | 4·57, | 4·67 | 1·14, | 0·70 |
| 6 | 2·73, | 2·98 | 4·09, | 3·63 | 1·36, | 0·65 |
| 7 | 2·42, | 3·61 | 4·34, | 4·61 | 1·92, | 1·00 |
| 8 | 2·61, | 2·54 | 3·73, | 3·60 | 1·12, | 1·06 |
| 9 | 2·58, | 2·98 | 4·22, | 4·55 | 1·64, | 1·57 |
| 10 | 3·75, | 3·60 | 5·08, | 4·50 | 1·33, | 0·90 |
| 11 | 2·4, | 2·4 | 3·66, | 3·7 | 1·26, | 1·3 |
| 12 | 2·7, | 2·5 | 3·6, | 2·7 | 0·9, | 0·2 |
| 13 | 3·64, | 3·32 | 4·35, | 4·27 | 0·71, | 0·95 |
| 14 | 2·69, | 3·40 | 5·83[d], | 8·49[d] | 3·14[c], | 5·09[c] |
| 15 | 3·05, | 3·41 | 3·89, | 3·79 | 0·84, | 0·38 |
| 16 | 3·7, | 4·2 | 4·4, | 4·2 | 0·7, | 0 |
| 17 | 3·27, | 1·37 | 4·98, | 2·29 | 1·71, | 0·92 |
| 18 | 1·7, | 3·6 | 2·5, | 2·5 | 0·8, | 1·0 |
| 19[b] | 3·0, | 4·0 | 4·0, | 6·0 | 1·0, | 2·0 |
| Mean ($\bar{x}$) | 3·08 | | 4·19 | | 1·04 | |
| Repeatability ($r$) | 1·55 | | 1·78 | | 1·11 | |
| Reproducibility ($R$) | 1·79 | | 2·04 | | 1·36 | |

For key, see Table XX.

## Organization of the Trial

METHODS

Participants were asked to use the three dietary fibre methods described above and a moisture procedure (drying to a constant weight at 103 ± 2°C in an atmospheric pressure drying oven) to enable all results to be expressed on a dry-matter basis.

LABORATORIES

A total of 18 analysts received samples and were able to attempt the requested analyses. Two of these analysts indicated that they would not be using the FMBRA pentose method.

*Samples*

Participants received samples in two stages, a pre-trial sample (wholemeal bread) and seven trial samples proper. The seven trial samples were subdivided to give 14 individual samples, each to be analysed once only, i.e. as blind duplicates.

TABLE VII

DIETARY FIBRE COLLABORATIVE TRIAL RESULTS FOR 1:1 MIX OF WHOLEMEAL (TABLES III/IV) AND WHITE (TABLES V/VI) BREADS BY ENGLYST GLC END-POINT METHOD
(Sample Code 5/9)

| | *g/100 g dry matter* | | | | | |
|---|---|---|---|---|---|---|
| Laboratory | Non-starch polysaccharides (NSP) | | NSP + resistant starch (RS) | | Resistant starch | |
| 1 | 7·25, | 7·11 | 8·13, | 8·30 | 1·03, | 1·13 |
| 2 | 7·39, | 6·32 | 8·37, | 7·35 | 1·03, | 1·19 |
| 3 | 7·79, | 7·77 | 9·40, | 9·68 | 1·42, | 1·39 |
| 4 | 6·83, | 7·05 | 7·51, | 7·86 | 0·82, | 0·81 |
| 5 | 7·48, | 8·22 | 8·27, | 11·49 | 0·80, | 1·93 |
| 6 | 6·54, | 5·98 | 7·27, | 7·25 | 0·98, | 1·06 |
| 7 | 5·50, | 7·92 | 6·46, | 9·86 | 1·08, | 1·58 |
| 8 | 6·01, | 6·01 | 7·34, | 6·46 | 1·07, | 0·83 |
| 9 | 7·23, | 7·77 | 8·07, | 7·39 | 0·93, | 0·43 |
| 10 | 6·10, | 5·63 | 6·82, | 5·69 | 0·91, | 0·34 |
| 11 | 5·08, | 6·54 | 5·34, | 7·20 | 0·71, | 0·75 |
| 12 | 11·6[d], | 12·6[d] | 10·8, | 9·4 | 0·1, | 0·1 |
| 13 | 7·65, | 6·76 | 6·80, | 8·04 | 0·06, | 0·75 |
| 14 | 7·51, | 6·11 | 7·92, | 8·81 | 1·58, | 2·02 |
| 15 | 5·36, | 5·47 | 7·75, | 6·52 | 1·62, | 0·91 |
| 16 | 7·2, | 8·5 | 7·3, | 6·9 | 0·7, | 0·8 |
| 17 | 6·16, | 7·04 | 6·63, | 8·70 | 0·71, | 1·27 |
| 18 | 3·5, | 5·7 | 6·46, | 5·4 | 1·3, | 0·3 |
| 19[b] | 8·0, | 4·0 | 16·0, | 5·9 | 4·1, | 1·7 |
| Mean ($\bar{x}$) | 6·66 | | 7·75 | | 0·96 | |
| Repeatability ($r$) | 2·20 | | 2·97 | | 1·02 | |
| Reproducibility ($R$) | 3·01 | | 3·95 | | 1·34 | |

For key, see Table XX.

The samples used were:

(a) Pre-trial: wholemeal bread
(b) Trial proper: wholemeal bread (coded 10 and 11, and same as pre-trial sample)

White bread (coded 4 and 13)
1:1 mix of wholemeal and white breads above (coded 5 and 9)
High bran bread (coded 2 and 14)
Rye bread (coded 6 and 8)
Cornflakes (coded 1 and 7)
Oat cereals (coded 3 and 12)

Each of the samples was commercially prepared, ground and homogenised before despatch to participants. They were all artifically coloured during preparation so as to appear identical.

## RESULTS

Each analyst received the 15 samples and was asked to carry out duplicate determinations on the pre-trial sample and single determinations on the 14 trial

TABLE VIII

DIETARY FIBRE COLLABORATIVE TRIAL RESULTS FOR 1:1 MIX OF WHOLEMEAL (TABLES III/IV) AND WHITE (TABLES V/VI) BREADS, BY ENGLYST COLORIMETRIC END-POINT METHOD
(Sample Code 5/9)

| | *g/100 g dry matter* | | | | | |
|---|---|---|---|---|---|---|
| Laboratory | Non-starch polysaccharides (NSP) | | NSP + resistant starch (RS) | | Resistant starch | |
| 1 | 7·36, | 7·10 | 8·64, | 7·96 | 1·28, | 0·86 |
| 2 | 8·06, | 7·75 | 8·47, | 9·15 | 0·41, | 1·35 |
| 3 | 8·03, | 8·46 | 9·30, | 9·92 | 1·65, | 1·46 |
| 4 | 8·62, | 7·04 | 8·64, | 7·71 | 0·02, | 0·67 |
| 5 | 8·30, | 8·91 | 9·22, | 9·62 | 0·92, | 0·71 |
| 6 | 6·75, | 6·60 | 7·90, | 7·19 | 1·15, | 0·59 |
| 7 | 5·62, | 7·95 | 5·79, | 9·61 | 0·17, | 1·66 |
| 8 | 6·11, | 6·28 | 7·73, | 6·87 | 1·62, | 0·59 |
| 9 | 7·03, | 7·86 | 8·59, | 9·07 | 1·56, | 1·21 |
| 10 | 8·86, | 8·28 | 9·20, | 9·05 | 0·34, | 0·77 |
| 11 | 7·3, | 4·8 | 8·16, | 7·0 | 0·73, | 2·2 |
| 12 | 5·2, | 6·4 | 4·8[d], | 5·3[d] | 0·1, | 0·1 |
| 13 | 7·80, | 7·99 | 8·91, | 9·42 | 1·11, | 1·43 |
| 14 | 7·70, | 7·60 | 8·14, | 9·27 | 0·44, | 1·67 |
| 15 | 7·43, | 7·43 | 7·58, | 8·47 | 0·15, | 1·04 |
| 16 | 8·6, | 8·1 | 9·3, | 9·3 | 0·7, | 1·2 |
| 17 | 7·53, | 4·50 | 8·33, | 6·51 | 0·80, | 2·01 |
| 18 | 7·7, | 10·3 | 8·5, | 11·6 | 0·8, | 1·3 |
| 19[b] | 11·0, | 6·0 | 15·0, | 8·0 | 4·0, | 2·0 |
| Mean ($\bar{x}$) | 7·43 | | 8·53 | | 0·97 | |
| Repeatability ($r$) | 2·73 | | 2·87 | | 1·63 | |
| Reproducibility ($R$) | 3·39 | | 3·15 | | 1·60 | |

For key, see Table XX.

samples proper. Analysts were asked to report results of the pre-trial sample before proceeding to the analysis of the remaining samples. For the two Englyst methods, analysts were asked to report:

(a) total NSP
(b) total NSP + resistant starch
(c) resistant starch content

The results obtained using the two Englyst procedures (i.e. with GLC or colorimetric end-point determinations) are given in Tables I–XVI.

For the FMBRA method, the final "dietary fibre" content is calculated from the determined pentose value using the given factor (see Appendix III). The results are given in Tables XVII–XIX.

STATISTICAL ANALYSES OF THE RESULTS

The results were analysed for outliers by the Cochran's and Dixon's Tests using procedures given by the British Standards Institution[19]. Some outlying

TABLE IX

DIETARY FIBRE COLLABORATIVE TRIAL RESULTS FOR HIGH BRAN BREAD BY ENGLYST GLC END-POINT METHOD

(Sample Code 2/14)

| Laboratory | *g/100 g dry matter* | | | | | |
|---|---|---|---|---|---|---|
| | Non-starch polysaccharides (NSP) | | NSP + resistant starch (RS) | | Resistant starch | |
| 1 | 11·20, | 11·59 | 11·98, | 12·20 | 0·72, | 0·68 |
| 2 | 10·26, | 10·57 | 10·96, | 11·20 | 0·51, | 0·72 |
| 3 | 13·06, | 12·86 | 14·16, | 13·89 | 0·97, | 0·83 |
| 4 | 10·49, | 12·10 | 13·78, | 13·32 | 1·33[d], | 1·74[d] |
| 5 | 11·74, | 12·73 | 13·03, | 13·60 | 0·47, | 1·00 |
| 6 | 9·57, | 9·73 | 10·03, | 9·50 | 0·49, | 0·31 |
| 7 | 8·16, | 11·79 | 8·69, | 13·27 | 0·74, | 1·15 |
| 8 | 9·23, | 9·44 | 9·32, | 9·47 | 0·52, | 0·41 |
| 9 | 11·02, | 9·38 | 10·50, | 10·46 | 0·47, | 0·63 |
| 10 | 10·00, | 11·10 | 10·15, | 9·85 | 0·46, | 0·49 |
| 11 | 9·50, | 8·52 | 10·23, | 9·38 | 0·58, | 0·51 |
| 12 | 8·4, | 9·9 | 6·0, | 9·6 | 0·1, | 0·1 |
| 13 | 7·87, | 11·65 | 8·18, | 13·32 | 0·30, | 0·91 |
| 14 | 11·53, | 10·92 | 12·17, | 12·18 | 1·77[d], | 1·74[d] |
| 15 | 10·53, | 9·65 | 9·68, | 10·07 | 0·46, | 0·52 |
| 16 | 24·1[c], | 8·7[c] | 24·7[c], | 9·9[c] | 1·2, | 0·5 |
| 17 | 10·44, | 10·68 | 11·37, | 11·30 | 0·70, | 0·59 |
| 18 | 10·2, | 9·4 | 11·3, | 9·2 | 1·2, | 0·5 |
| 19[b] | 9·2, | — | 14·0, | — | 1·9, | — |
| Mean ($\bar{x}$) | 10·45 | | 10·98 | | 0·62 | |
| Repeatability ($r$) | 3·08 | | 3·96 | | 0·70 | |
| Reproducibility ($R$) | 3·75 | | 5·40 | | 0·79 | |

For key, see Table XX.

results were identified and these were not used in the calculations of means ($\bar{x}$), repeatabilities ($r$) or reproducibilities ($R$).

In addition, the results from one laboratory were not used in any of the calculations as that laboratory clearly encountered substantial analytical difficulties.

The final values of the means, repeatabilities and reproducibilities are given in Tables I–XIX, and collated for convenience in Tables XXI–XXIII. The values are as defined and calculated by the procedures described by the British Standards Institution[19].

## Discussion

From the results of the collaborative trial, a comparison may be made between the three methods being tested. Comparison of the mean values obtained for total NSP and total NSP with resistant starch by the two Englyst methods indicates that there is no significant statistical difference between the means obtained by each method, though the values obtained with the colorimetric end-point are always slightly greater than those obtained by the GLC end-point.

TABLE X

DIETARY FIBRE COLLABORATIVE TRIAL RESULTS FOR HIGH BRAN BREAD BY ENGLYST COLORIMETRIC END-POINT METHOD
(Sample Code 2/14)

| Laboratory | *g/100 g dry matter* | | | | | |
|---|---|---|---|---|---|---|
| | Non-starch polysaccharides (NSP) | | NSP + resistant starch (RS) | | Resistant starch | |
| 1 | 11·45, | 11·32 | 12·03, | 12·30 | 0·58, | 0·98 |
| 2 | 11·21, | 11·80 | 11·88, | 12·60 | 0·67, | 0·80 |
| 3 | 13·21, | 13·20 | 13·75, | 13·89 | 0·54, | 0·69 |
| 4 | 14·17, | 12·79 | 12·54, | 13·07 | 0, | 0·28 |
| 5 | 12·16, | 13·44 | 13·94, | 13·28 | 1·78, | 0 |
| 6 | 10·34, | 10·20 | 10·35, | 10·50 | 0·01, | 0·30 |
| 7 | 7·89[c], | 12·51[c] | 8·80, | 13·71 | 0·91, | 1·20 |
| 8 | 9·51, | 9·38 | 9·78, | 10·26 | 0·27, | 0·88 |
| 9 | 11·06, | 11·67 | 12·57, | 13·03 | 1·51, | 1·36 |
| 10 | 13·34, | 12·61 | 13·73, | 13·49 | 0·39, | 0·88 |
| 11 | 9·4, | 10·2 | 10·7, | 10·4 | 1·3, | 0·2 |
| 12 | 7·1[c], | 15·4[c] | 6·3, | 12·1 | 0·1, | 0·1 |
| 13 | 9·05, | 9·00 | 9·24, | 9·20 | 0·19, | 0·20 |
| 14 | 11·22, | 10·78 | 12·92, | 13·61 | 1·70, | 2·83 |
| 15 | 11·19, | 12·37 | 10·94, | 13·27 | −0·25[e], | 0·90 |
| 16 | 14·0, | 12·9 | 15·9, | 13·2 | 1·9, | 0·3 |
| 17 | 10·63[c], | 3·73[c] | 11·88, | 7·67 | 1·25, | 3·94 |
| 18 | 9·8[c], | 12·6[c] | 11·0, | 13·2 | 1·2, | 0·5 |
| 19[b] | 11·0, | 14·0 | 13·0, | 16·0 | 2·0, | 2·0 |
| Mean ($\bar{x}$) | 11·53 | | 11·85 | | 0·85 | |
| Repeatability ($r$) | 1·54 | | 4·60 | | 1·99 | |
| Reproducibility ($R$) | 4·40 | | 5·70 | | 2·38 | |

For key, see Table XX.

If a comparison is made between the repeatabilities and reproducibilities for samples obtained by each Englyst method, then no consistent pattern emerges; the two methods have, within experimental error, the same precision parameters.

The choice between the two Englyst methods is dependent only on the circumstances in which they are to be used. It is suggested that the procedure with the GLC end-point may be regarded as the reference method, giving detailed information on the specific sugar composition of the dietary fibre fraction of a sample, and that the colorimetric method be regarded as a "quality

control" method, which may be assumed to give equivalent (though less detailed) results to the reference method.

The actual values of repeatability and reproducibility obtained for either Englyst method are considered to be satisfactory for this particular determination. In particular, they are similar for cereal samples to those obtained in recent trials of the method of Prosky *et al.* and which has now been adopted by the Association of Official Analytical Chemists (AOAC)[20,21].

TABLE XI

DIETARY FIBRE COLLABORATIVE TRIAL RESULTS FOR RYE BREAD BY ENGLYST GLC END-POINT METHOD
(Sample Code 6/8)

| Laboratory | g/100 g dry matter | | | | | |
|---|---|---|---|---|---|---|
| | Non-starch polysaccharides (NSP) | | NSP + resistant starch (RS) | | Resistant starch | |
| 1 | 4·60, | 4·65 | 5·75, | 5·90 | 1·32, | 1·34 |
| 2 | 4·91, | 4·40 | 6·04, | 5·69 | 1·46, | 1·43 |
| 3 | 5·19, | 5·13 | 6·79, | 6·90 | 1·62, | 1·72 |
| 4 | 3·88, | 4·38 | 5·58, | 5·79 | 1·36, | 1·31 |
| 5 | 4·76, | 3·30 | 6·36, | 6·04 | 1·38, | 2·43 |
| 6 | 3·91, | 4·04 | 5·24, | 5·23 | 1·35, | 1·29 |
| 7 | 5·08, | 5·10 | 6·55, | 6·39 | 1·59, | 1·49 |
| 8 | 3·94, | 3·90 | 4·96, | 5·09 | 1·18, | 1·25 |
| 9 | 4·96, | 4·81 | 6·71, | 6·94 | 1·60, | 1·71 |
| 10 | 3·41, | 2·90 | 4·51, | 3·80 | 1·15, | 0·88 |
| 11 | 3·63, | 3·00 | 5·17, | 4·63 | 1·52, | 1·39 |
| 12 | 9·8[c], | 7·1[c] | 7·9[c], | 4·2[c] | 1·1, | 0·1 |
| 13 | 4·07, | 4·55 | 5·21, | 4·81 | 1·17, | 0·80 |
| 14 | 4·81, | 5·61 | 7·35, | 7·56 | 2·40[d], | 2·51[d] |
| 15 | 2·54, | 3·46 | 5·03, | 5·37 | 1·50, | 1·50 |
| 16 | 3·8, | 3·1 | 4·1, | 4·4 | 0·7, | 1·1 |
| 17 | 3·87, | 3·86 | 4·97, | 4·82 | 1·15, | 1·06 |
| 18 | 2·8, | 3·0 | 4·09, | 4·3 | 0·9, | 1·2 |
| 19[b] | 5·0, | 5·0 | 6·5, | 9·1 | 3·4, | 6·2 |
| Mean ($\bar{x}$) | 4·10 | | 5·53 | | 1·30 | |
| Repeatability ($r$) | 1·15 | | 0·63 | | 0·79 | |
| Reproducibility ($R$) | 2·30 | | 2·82 | | 1·08 | |

For key, see Table XX.

Study of the repeatabilities and reproducibilities obtained when resistant starch alone is calculated shows that the variation in the assay of this parameter is excessive. It is therefore recommended that there be no requirement that resistant starch values alone be claimed.

Comparison between the results from samples analysed by the FMBRA method and the Englyst procedures shows that the methods will give equivalent mean values for wheat-based samples, though the FMBRA procedure yields somewhat lower values than either Englyst method. This may be due to incomplete digestion within the method or an inappropriate conversion factor

being used in the pentose/dietary fibre calculation. The precision characteristics of the three methods are similar, though the values of reproducibility for the FMBRA procedure are somewhat higher than those obtained for the Englyst methods.

The results of the trial show that the range of samples to which the FMBRA method may be applied is limited. The values obtained for the cornflakes and oat cereal samples are, as expected, much lower than the "true" dietary fibre content values.

TABLE XII

DIETARY FIBRE COLLABORATIVE TRIAL RESULTS FOR RYE BREAD BY ENGLYST COLORIMETRIC END-POINT METHOD

(Sample Code 6/8)

| Laboratory | *g/100 g dry matter* | | | | | |
|---|---|---|---|---|---|---|
| | Non-starch polysaccharides (NSP) | | NSP + resistant starch (RS) | | Resistant starch | |
| 1 | 4·99, | 4·86 | 6·22, | 6·14 | 1·23, | 1·28 |
| 2 | 5·22, | 5·13 | 6·48, | 6·15 | 1·26, | 1·02 |
| 3 | 5·36, | 5·62 | 6·46, | 6·72 | 1·10, | 1·10 |
| 4 | 5·14, | 3·37 | 6·28, | 5·21 | 1·14, | 1·84 |
| 5 | 4·94, | 5·36 | 6·76, | 7·38 | 1·82, | 2·02 |
| 6 | 4·46, | 4·54 | 5·17, | 5·28 | 0·71, | 0·74 |
| 7 | 4·85, | 5·01 | 6·36, | 6·32 | 1·51, | 1·31 |
| 8 | 4·07, | 4·05 | 5·07, | 5·22 | 1·00, | 1·17 |
| 9 | 4·62, | 4·71 | 6·32, | 6·23 | 1·70, | 1·52 |
| 10 | 5·91, | 5·18 | 7·04, | 6·07 | 1·13, | 0·89 |
| 11 | 3·56, | 4·01 | 4·9, | 4·5 | 1·34, | 0·49 |
| 12 | 2·8, | 4·7 | 2·8[d], | 4·3[d] | 0·1, | 0·1 |
| 13 | 5·04, | 5·09 | 6·27, | 6·07 | 1·23, | 0·98 |
| 14 | 3·88, | 5·28 | 7·24, | 8·00 | 3·36[d], | 2·71[d] |
| 15 | 4·57, | 4·55 | 5·84, | 6·15 | 1·27, | 1·60 |
| 16 | 5·4, | 5·3 | 6·2, | 5·8 | 0·7, | 0·5 |
| 17 | 4·98, | 3·06 | 5·52, | 6·03 | 0·54[c], | 2·97[c] |
| 18 | 4·7, | 5·3 | 5·6, | 5·8 | 0·9, | 0·6 |
| 19[b] | 4·0, | 4·0 | 6·0, | 7·0 | 2·0, | 3·0 |
| Mean ($\bar{x}$) | 4·71 | | 6·08 | | 1·10 | |
| Repeatability ($r$) | 1·75 | | 0·97 | | 0·67 | |
| Reproducibility ($R$) | 2·02 | | 2·10 | | 1·32 | |

For key, see Table XX.

Nevertheless, the method can be recommended as a "quality control" procedure when applied to the appropriate samples.

Comparison of the mean values obtained by all three methods studied in the trial on the wholemeal and white breads, and the 1 : 1 mixed bread sample prepared from these two breads, indicates that the methods are giving good recoveries. The mean value determined for the mixed sample is only slightly greater than the value calculated from the analysis of the individual samples.

The Englyst procedure is able to measure dietary fibre with or without resistant starch. The authors believe that dietary fibre should be defined and measured as non-starch polysaccharides and not NSP plus RS. Since recent

studies[10,11,12] have shown that RS is only a small proportion of the starch escaping digestion in the small intestine no sound reason can be seen for the measurement of NSP together with RS.

The Englyst procedure is considered to be more appropriate than the AOAC procedure for the measurement of dietary fibre, as it is more specific and gives more detailed results. Further, dietary fibre measured as NSP is independent of food processing and treatment applied to the sample before analysis.

TABLE XIII

DIETARY FIBRE COLLABORATIVE TRIAL RESULTS FOR CORNFLAKES BY ENGLYST GLC END-POINT METHOD

(Sample Code 1/7)

| | *g/100 g dry matter* | | | | | |
|---|---|---|---|---|---|---|
| Laboratory | Non-starch polysaccharides (NSP) | | NSP + resistant starch (RS) | | Resistant starch | |
| 1 | 0·60, | 0·57 | 3·57, | 3·43 | 2·96, | 2·84 |
| 2 | 0·87, | 0·89 | 4·12, | 4·31 | 3·25, | 3·37 |
| 3 | 1·01, | 1·09 | 4·32, | 4·48 | 3·30, | 3·38 |
| 4 | 0·58, | 0·83 | 5·25, | 5·42 | 4·58, | 4·53 |
| 5 | 0·82, | 0·48 | 3·65, | 3·90 | 2·90, | 3·43 |
| 6 | 0·73, | 0·58 | 4·31, | 4·66 | 3·54, | 3·96 |
| 7 | 1·41, | 1·01 | 4·85, | 4·44 | 3·46, | 3·43 |
| 8 | 0·54, | 0·57 | 3·69, | 3·57 | 3·12, | 3·00 |
| 9 | 1·61, | 1·27 | 4·36, | 4·20 | 2·97, | 2·96 |
| 10 | 0·52, | 0·53 | 3·41, | 3·10 | 2·93, | 2·54 |
| 11 | 2·48[c], | 0·61[c] | 3·81, | 4·27 | 0·95, | 2·71 |
| 12 | 3·8[c], | 2·2[c] | 4·5, | 7·1 | 1·7[c], | 5·3[c] |
| 13 | 1·16, | 0·75 | 3·72, | 3·35 | 2·74, | 2·60 |
| 14 | 1·31, | 2·01 | 12·09[d], | 11·15[d] | 10·74[c], | 9·76[c] |
| 15 | 1·08, | 0·45 | 5·50, | 4·09 | 4·87, | 3·45 |
| 16 | 0·5, | 0·6 | 2·4, | 4·4 | 1·8, | 3·8 |
| 17 | 0·49, | 0·54 | 3·40, | 3·74 | 2·93, | 3·16 |
| 18 | 0·4, | 0·05 | 5·4, | 3·5 | 5·2, | 3·4 |
| 19[b] | 14·0, | 4·0 | 23·0, | 7·9 | 9·0, | 3·9 |
| Mean ($\bar{x}$) | 0·81 | | 4·18 | | 3·25 | |
| Repeatability ($r$) | 0·65 | | 2·02 | | 1·81 | |
| Reproducibility ($R$) | 1·16 | | 2·46 | | 2·32 | |

For key, see Table XX.

In contrast to the Englyst procedure, the value for dietary fibre obtained by the AOAC method[20,21] will vary depending on food processing and pre-treatment of samples for analysis. This makes the AOAC method unusable as a means of enforcing labelling regulations for dietary fibre.

It has been suggested that the residual starch that is included as dietary fibre in the AOAC (1985) analysis reflects the amount escaping digestion in the small intestine. This has not been proved and, from a number of experimental studies in man, it is clear that much greater amounts of starch escape digestion in the small intestine than can be accounted for by the AOAC procedure. The dietary fibre value obtained by the AOAC (1985) procedure is not a measure of any

definable component of the diet, such as NSP, nor does it represent NSP plus starch escaping digestion in the small intestine of man.

Methods for measuring dietary fibre that include starch in any form are fundamentally in error and likely to lead to confusion and disputes. If starch is accepted as dietary fibre, then food manufacturers will be able to increase the dietary fibre content of starchy foods in a way that will undermine the true value of dietary fibre as a food component.

TABLE XIV

DIETARY FIBRE COLLABORATIVE TRIAL RESULTS FOR CORNFLAKES BY ENGLYST COLORIMETRIC END-POINT METHOD

(Sample Code 1/7)

| | *g/100 g dry matter* | | | | | |
|---|---|---|---|---|---|---|
| Laboratory | Non-starch polysaccharides (NSP) | | NSP + resistant starch (RS) | | Resistant starch | |
| 1 | 1·11, | 1·22 | 3·79, | 3·81 | 2·68, | 2·59 |
| 2 | 1·15, | 1·51 | 4·16, | 4·52 | 3·01, | 3·01 |
| 3 | 1·69, | 1·7 | 4·70, | 4·59 | 3·01, | 2·88 |
| 4 | 1·91, | 0·89 | 3·93, | 1·59 | 2·02, | 0·70 |
| 5 | 1·24, | 1·19 | 4·12, | 4·16 | 2·88, | 2·97 |
| 6 | 0·49, | 0·64 | 3·90, | 3·85 | 3·41, | 3·21 |
| 7 | 1·33, | 1·08 | 4·17, | 4·22 | 2·84, | 3·14 |
| 8 | 0·78, | 0·58 | 3·73, | 3·68 | 2·95, | 3·10 |
| 9 | 0·85, | 0·85 | 3·89, | 3·88 | 3·04, | 3·03 |
| 10 | 1·15, | 1·39 | 4·43, | 4·82 | 3·28, | 3·43 |
| 11 | 1·6, | 0·70 | 4·3, | 3·00 | 2·7, | 2·30 |
| 12 | 3·7[c], | 1·6[c] | 3·9, | 2·3 | 0·2[d], | 0·7[d] |
| 13 | 1·18, | 1·27 | 4·15, | 4·00 | 2·97, | 2·73 |
| 14 | 1·24, | 1·60 | 12·49[d], | 11·33[d] | 11·25[d], | 9·73[d] |
| 15 | 0·95, | 1·14 | 3·27, | 3·61 | 2·32, | 2·47 |
| 16 | 1·0, | 0·7 | 3·6, | 4·7 | 2·6, | 4·0 |
| 17 | 1·41, | 0·72 | 2·72, | 3·82 | 1·31, | 3·10 |
| 18 | n/d | 0·8 | 4·5, | 4·4 | 4·5, | 3·6 |
| 19[b] | 6·0, | 2·0 | 9·0, | 4·0 | 3·0, | 2·0 |
| Mean ($\bar{x}$) | 1·09 | | 3·89 | | 2·87 | |
| Repeatability ($r$) | 0·91 | | 1·72 | | 1·43 | |
| Reproducibility ($R$) | 1·14 | | 1·97 | | 1·95 | |

For key, see Table XX.

A few technical modifications have been introduced in the Englyst procedure since the present trial was initiated. These changes have reduced the analysis time but do not affect the results obtained or the accuracy of the method. The Englyst procedure, including the latest modifications, the measurement of soluble and insoluble NSP and with both the GLC and colorimetric options, is described elsewhere[15].

## Conclusion

The results of this trial indicate that the Englyst method with GLC end-point determination may be recommended as the reference method for the

determination of dietary fibre in the U.K. The method may be adjusted to provide an analytical result that equates to total non-starch polysaccharide content or total non-starch polysaccharide and resistant starch content. The method is not suitable for the evaluation of the resistant starch content of a sample.

The results also indicate that the other two methods evaluated, i.e. the Englyst procedure with colorimetric end-point determination and the FMBRA

TABLE XV
DIETARY FIBRE COLLABORATIVE TRIAL RESULTS FOR OAT CEREAL BY ENGLYST GLC END-POINT METHOD
(Sample Code 3/12)

| | *g/100 g dry matter* | | | | | |
|---|---|---|---|---|---|---|
| Laboratory | Non starch polysaccharides (NSP) | | NSP + resistant starch (RS) | | Resistant starch | |
| 1 | 8·34, | 8·42 | 8·18, | 8·36 | −0·16,[e] | 0·17 |
| 2 | 8·80, | 8·31 | 9·88, | 8·29 | 0·64,[c] | 0·10[c] |
| 3 | 8·91, | 9·14 | 10·05, | 9·79 | 0·62, | 0·55 |
| 4 | 9·48, | 8·05 | 9·37, | 8·01 | 0·03, | 0 |
| 5 | 8·74, | 9·04 | 8·58, | 9·08 | 0·03, | 0·02 |
| 6 | 7·75, | 7·69 | 7·98, | 7·48 | 0·11, | −0·13[e] |
| 7 | 3·33,[c] | 9·17[c] | 3·12, | 9·16 | 0·09, | −0·14[e] |
| 8 | 7·27, | 7·57 | 7·59, | 7·59 | 0·31,[c] | 0[c] |
| 9 | 8·93, | 8·85 | 8·77, | 8·41 | −0·38,[e] | −0·34[e] |
| 10 | 7·59, | 6·81 | 7·07, | 6·67 | −0·29,[e] | −0·33[e] |
| 11 | 7·34, | 7·11 | 7·15, | 6·49 | −0·01,[e] | −0·38[e] |
| 12 | 10·6, | 8·0 | 6·7, | 8·2 | <0·1, | 0·1 |
| 13 | 9·62, | 8·24 | 11·84, | 7·69 | 0·91,[c] | −0·64[e,c] |
| 14 | 9·05, | 8·85 | 12·16, | 12·61 | 3·47,[d] | 3·60[d] |
| 15 | 7·14, | 7·63 | 8·31, | 8·62 | 0·71, | 0·65 |
| 16 | 9·5, | 8·2 | 11·1, | 7·4 | 0·4, | 0·4 |
| 17 | 6·30, | 8·26 | 6·52, | 9·20 | 0·16,[c] | 0·82[c] |
| 18 | 7·2, | 7·2 | 9·9, | 6·8 | 1·9,[c] | −0·2[e,c] |
| 19[b] | 7·0, | — | 8·0, | 15·0 | 2·0, | – |
| Mean ($\bar{x}$) | 8·23 | | 8·45 | | 0·17 | |
| Repeatability ($r$) | 2·04 | | 4·53 | | 0·14 | |
| Reproducibility ($R$) | 2·67 | | 5·12 | | 0·70 | |

For key, see Table XX.

method, are suitable for use as quality control methods, provided that the applicability of the FMBRA method, in particular, was confirmed. This method has been designed to be used with wheat products.

The Englyst procedures have been applied to the analysis of non-cereal product samples; it is suggested that their applicability to such samples be confirmed by carrying out further trials using a range of non-cereal foods.

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TABLE XVI

DIETARY FIBRE COLLABORATIVE TRIAL RESULTS FOR OAT CEREAL BY ENGLYST COLORIMETRIC END-POINT METHOD

(Sample Code 3/12)

| Laboratory | *g/100 g dry matter* | | | | | |
|---|---|---|---|---|---|---|
| | Non starch polysaccharides (NSP) | | NSP + resistant starch (RS) | | Resistant starch | |
| 1 | 8·63, | 8·46 | 8·68, | 8·48 | 0·05, | 0·02 |
| 2 | 9·48, | 9·97 | 9·55, | 9·91 | 0·07, | 0·0 |
| 3 | 9·48, | 9·51 | 10·02, | 10·05 | 0·54, | 0·54 |
| 4 | 8·64, | 8·53 | 7·98, | 8·18 | 0·0, | 0·0 |
| 5 | 9·19, | 8·98 | 9·58, | 9·38 | 0·39, | 0·40 |
| 6 | 8·11, | 7·80 | 8·13, | 7·24 | 0·02, | −0·56[e] |
| 7 | 3·48,[c] | 8·34[c] | 3·58,[c] | 8·67[c] | 0·1, | 0·33 |
| 8 | 7·24, | 7·74 | 7·91, | 7·80 | 0·67, | 0·06 |
| 9 | 8·61, | 8·73 | 9·86, | 9·25 | 1·25, | 0·52 |
| 10 | 9·73, | 9·34 | 9·97, | 9·65 | 0·24, | 0·31 |
| 11 | 7·3, | 7·8 | 7·5, | 8·2 | 0·5, | 0·4 |
| 12 | 7·1, | 4·4 | 6·6, | 6·8 | <0·1,[c] | 2·4[c] |
| 13 | 11·70, | 12·28 | 12·99, | 11·80 | 1·29, | 1·05 |
| 14 | 9·10, | 10·16 | 12·44, | 13·06 | 3·34,[d] | 2·90[d] |
| 15 | 8·60, | 8·83 | 7·63, | 8·73 | −0·97,[e] | −0·10[e] |
| 16 | 10·8, | 9·7 | 11·4, | 9·9 | 0·6, | 0·2 |
| 17 | 8·06, | 5·44 | 8·82,[c] | 5·88[c] | 0·76, | 0·44 |
| 18 | 6·0,[c] | 12·8[c] | 7·5,[c] | 12·0[c] | 1·5,[c] | −0·8[c,e] |
| 19[b] | 4·0, | 9·0 | 7·0, | 10·0 | 3·0, | 1·0 |
| Mean ($\bar{x}$) | 8·73 | | 9·30 | | 0·36 | |
| Repeatability ($r$) | 2·13 | | 1·36 | | 0·59 | |
| Reproducibility ($R$) | 4·43 | | 4·93 | | 1·06 | |

For key, see Table XX.

Bostock Hill and Rigby, Birmingham; A. Harrison, County of Avon Scientific Services, Bristol; G. Holcombe, Laboratory of the Government Chemist, London; S. Landsman, Moir and Palgrave, London; A. Law, RHM, High Wycombe; G. Meadows, Lincolne Sutton and Wood, Norwich; R. Mongeau, Health and Welfare, Ottawa, Canada; L. Perkin, West Yorkshire County Analyst, Wakefield; A. Phillips, City Analyst's Laboratory, Cardiff; J. Shelton, Leo Taylor and Lucke, London; A.-M. Sorensen, National Food Laboratory, Copenhagen, Denmark; D. Southgate, Institute of Food Research, Norwich.

TABLE XVII

DIETARY FIBRE COLLABORATIVE TRIAL RESULTS BY THE FMBRA METHOD

| Laboratory | Dietary fibre (*g/100 g dry matter*) | | | | | |
|---|---|---|---|---|---|---|
| | Wholemeal bread pre-trial sample (same as Sample 10/11) | | Wholemeal bread Sample 10/11 (same as pre-trial sample) | | White bread Sample 4/13 | |
| 1 | —[a] | | —[a] | | —[a] | |
| 2 | 10·5, | 10·7 | 9·81, | 10·05 | 2·42, | 2·86 |
| 3 | 11·12, | 11·31 | 8·52, | 8·50 | 3·91, | 3·52 |
| 4 | 9·51, | 8·93 | 8·39, | 7·78 | 1·27, | 2·01 |
| 5 | 10·5, | 10·7 | 12·69, | 12·50 | 2·02, | 1·81 |
| 6 | 9·21, | 10·34 | 8·98, | 7·83 | 0·99, | 2·09 |
| 7 | 8·24, | 8·33 | 7·48, | 6·81 | 1·84, | 1·23 |
| 8 | 10·31, | 10·12 | 8·99, | 9·99 | 1·84, | 1·95 |
| 9 | —[a] | | —[a] | | —[a] | |
| 10 | 10·3, | 10·3 | 7·32, | 8·27 | 1·65, | 1·34 |
| 11 | 11·4, | 13·3 | 10·2, | 9·3 | 3·8, | 4·3 |
| 12 | 10·5, | 11·0 | 9·5, | 8·4 | 3·6, | 1·9 |
| 13 | 11·60, | 10·93 | 8·46, | 8·95 | 2·00, | 1·35 |
| 14 | 10·64, | 10·87 | 6·27, | 6·11 | 2·04, | 1·68 |
| 15 | 12·33, | 10·87 | 8·06, | 8·53 | 1·84, | 2·18 |
| 16 | —[a] | | 11·7, | 9·9 | 3·1, | 0·2 |
| 17 | 10·06, | 10·40 | 7·50, | 5·86 | 4·07, | 2·14 |
| 18 | —[a] | | 9·2, | 8·9 | 2·1, | 2·5 |
| 19[b] | 17·4, | 16·0 | 14·0, | 7·0 | 2·0, | 4·0 |
| Mean ($\bar{x}$) | 10·51 | | 8·77 | | 2·24 | |
| Repeatability ($r$) | 1·55 | | 1·78 | | 2·16 | |
| Reproducibility ($R$) | 3·05 | | 4·63 | | 2·73 | |

For key, see Table XX.

## References


1. Cummings, J. H., Englyst, H. N., and Wood, R., *J. Assoc. Publ. Analysts*, 1985, **23**, 1.
2. Englyst, H. N., Cummings, J. H., and Wood, R., *J. Assoc. Publ. Analysts*, 1987, **25**, 59–71.
3. "Draft MAFF Guidelines on Nutrition Labelling". Ministry of Agriculture, Fisheries and Food, London, February 1986.
4. Bell, B. M., *J. Sci. Food Agric.*, 1985, **36**, 815.
5. Trowell, H., *Atherosclerosis* 1972, **16**, 138–40.
6. Trowell, H. In: "Dietary fibre, fibre-depleted foods and disease" (H. Trowell, D. Burkitt and K. Heaton, Eds.), Academic Press, London 1985, pp. 1–20.
7. Englyst, H., Wiggins, H. S., and Cummings, J. H., *Analyst*, 1982, **107**, 307–318.
8. Englyst, H. H., and Cummings, J. H. In: "Cereals in a European context" (Morton, I. D., Ed.). Ellis Horwood Ltd., Chichester 1987, pp. 221–233.
9. Englyst, H. N., Anderson, V., and Cummings, J. H., *J. Sci. Food Agric.*, 1983, **34**, 1434–1440.
10. Englyst, H. N., and Cummings, J. H., *Am. J. Clin. Nutr.*, 1985, **42**, 778–787.
11. Englyst, H. N., and Cummings, J. H., *Am. J. Clin. Nutr.*, 1986, **44**, 42–50.
12. Englyst, H. N., and Cummings, J. H., *Am. J. Clin. Nutr.*, 1987, **45**, 423–431.
13. Hartley, R. D., *Am. J. Clin. Nutr.*, 1978, **31**, S90.
14. Englyst, H. N., and Cummings, J. H., *Analyst*, 1984, **109**, 937–942.
15. Englyst, H. N., and Cummings, J. H., *J. Assoc. Off. Anal. Chem.*, 1987 (*in press*).
16. Dahlqvist, A., *J. Clin. Lab. Invest.*, 1962, **14**, 145–151.
17. Englyst, H. N., and Hudson, G. J., *Food Chem.*, 1987, **24**, 63–76.

18. Southgate, D. A. T., "Determination of Food Carbohydrates". Applied Sci. Publ., London 1976, pp. 137–141.
19. British Standards Institution, "Precision of Test Methods", BS 5497: Part I London, 1979.
20. Prosky, L., Asp, N. H., Furda, I., Devries, J. W., Schweizer, T. F., and Harland, B. F., *J. Assoc. Off. Anal. Chem.*, 1984, **67**, 1044–1052.
21. Prosky, L., Asp, N. G., Furda, I., Devries, J. W., Schweizer, T. F., and Harland, B. F., *J. Assoc. Off. Anal. Chem.*, 1985, **68**, 677–679.

TABLE XVIII

DIETARY FIBRE COLLABORATIVE TRIAL RESULTS BY THE FMBRA METHOD

| Laboratory | Dietary fibre (*g/100 g dry matter*) 1 : 1 mix wholemeal white breads Sample 5/9 | | High bran bread Sample 2/14 | | Dry bread Sample 6/8 | |
|---|---|---|---|---|---|---|
| 1 | —[a] | | —[a] | | —[a] | |
| 2 | 7·12, | 7·68 | 10·55, | 10·74 | 4·33, | 4·18 |
| 3 | 8·37, | 8·41 | 13·71, | 14·55 | 5·17, | 4·87 |
| 4 | 7·62, | 5·97 | 11·05, | 7·05 | 2·23, | 3·66 |
| 5 | 8·13, | 7·90 | 14·97, | 13·25 | 3·33, | 4·08 |
| 6 | 5·03, | 4·94 | 10·00, | 10·16 | 2·79, | 2·47 |
| 7 | 4·55, | 5·06 | 10·51, | 10·16 | 3·37, | 2·51 |
| 8 | 6·78, | 6·28 | 9·25, | 11·06 | 3·61, | 3·37 |
| 9 | —[a] | | —[a] | | —[a] | |
| 10 | 6·45, | 5·68 | 10·02, | 9·42 | 3·59, | 2·68 |
| 11 | 6·9, | 5·9 | 8·4 | 7·8 | 3·9, | 3·8 |
| 12 | 9·4, | 7·8 | 10·2, | 8·8 | 5·4, | 4·9 |
| 13 | 7·53, | 6·39 | 11·35, | 9·89 | 3·09, | 2·37 |
| 14 | 5·57, | 3·93 | 9·53, | 7·05 | 2·82, | 2·58 |
| 15 | 4·98, | 6·01 | 9·41, | 10·99 | 3·03, | 3·19 |
| 16 | 6·2, | 8·3 | 10·0, | 11·3 | 6·0, | 7·4 |
| 17 | 8·28, | 5·71 | 12·89, | 12·31 | 4·42, | 3·63 |
| 18 | 4·7, | 6·0 | 10·7, | 11·4 | 3·2, | 4·0 |
| 19[b] | 12·0, | 11·0 | 15·0, | 13·0 | 6·0, | 7·0 |
| Mean ($\bar{x}$) | 6·55 | | 10·58 | | 3·75 | |
| Repeatibility ($r$) | 2·53 | | 3·13 | | 1·46 | |
| Reproducibility ($R$) | 3·93 | | 5·41 | | 3·28 | |

For key, see Table XX.

## APPENDIX I

## ENGLYST PROCEDURE FOR DETERMINATION OF DIETARY FIBRE AS NON-STARCH POLYSACCHARIDES: MEASUREMENT OF CONSTITUENT SUGARS BY GAS-LIQUID CHROMATOGRAPHY

### 1. SCOPE AND FIELD OF APPLICATION

The method determines dietary fibre as non-starch polysaccharides (NSP).

### 2. DEFINITION

The content of dietary fibre: the content of non-starch polysaccharides as determined by the method specified.

TABLE XIX
DIETARY FIBRE COLLABORATIVE TRIAL RESULTS BY THE FMBRA METHOD

| Laboratory | Dietary fibre (*g/100 g dry matter*) Cornflakes Sample 1/7 | | Oat cereal Sample 3/12 | |
|---|---|---|---|---|
| 1 | —[a] | | —[a] | |
| 2 | 0·0, | 0·0, | 3·95, | 3·82 |
| 3 | 0·09, | 0·09 | 5·83,[d] | 5·96[d] |
| 4 | 0·0, | 0·0, | 2·45, | 2·62 |
| 5 | 0·0, | 0·0, | 3·28, | 3·45 |
| 6 | −0·84,[e] | −0·61[e] | 1·79, | 2·60 |
| 7 | −0·81,[e] | −0·80[e] | 3·38, | 1·79 |
| 8 | — | — | 2·82, | 2·97 |
| 9 | —[a] | | —[a] | |
| 10 | nil | nil | 2·51, | 2·33 |
| 11 | nil | nil | 2·9, | 3·0 |
| 12 | <0·1,[c] | 2·5[c] | 4·0, | 3·6 |
| 13 | 0·0, | 0·0, | 4·03, | 3·24 |
| 14 | 0·0, | 0·0, | 2·47, | 1·86 |
| 15 | −0·58,[e] | −0·39[e] | 2·42, | 2·48 |
| 16 | 0·0, | 0·0, | 3·9, | 5·2 |
| 17 | −0·31,[e] | −0·36[e] | 3·58, | 1·95 |
| 18 | n/d | n/d | 3·1, | 1·4 |
| 19[b] | 1·4, | 5·0 | 7·0, | 3·0 |
| Mean ($\bar{x}$) | 0·01 | | 2·96 | |
| Repeatability ($r$) | 0·00 | | 1·77 | |
| Reproducibility ($R$) | 0·07 | | 2·40 | |

For key, see Table XX.

## 3. PRINCIPLE

The starch is gelatinised and then removed by enzymic digestion. The remaining polysaccharides are hydrolysed by sulphuric acid and the resulting individual neutral sugars are measured by gas–liquid chromatography (GLC) of their alditol acetate derivatives. Uronic acids are measured separately by a colorimetric procedure. Two alternative procedures are described for the dispersion of the starch thus enabling evaluation of the sample's resistant starch content if required.

## 4 REAGENTS

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

TABLE XX
DIETARY FIBRE COLLABORATIVE TRIAL RESULTS
KEY TO TABLES I–XIX

a. Analysis not carried out by Laboratory.
b. Laboratory results not included in any calculations of mean, repeatability or reproducibility.
c. Rejected by Cochran's Test $P \leqslant 0{\cdot}05$. Values not used in calculations.
d. Rejected by Dixon's Test $P \leqslant 0{\cdot}05$. Values not used in calculations.
e. Results reported as negative, considered as zero (0·00) in calculations.
n/d: not detected, considered as zero (0·00) in calculations.

4.1 *Acetone*

4.2 *Dimethyl Sulphoxide (DMSO)*

4.3 *Benzoic Acid Solution, 50 per cent. saturated*

Dilute saturated benzoic acid solution 1 : 1 v/v with water.

TABLE XXI

DIETARY FIBRE COLLABORATIVE TRIAL SUMMARY OF MEANS, REPEATABILITIES AND REPRODUCIBILITIES FOR THREE METHODS

| Method | | Sample | | |
|---|---|---|---|---|
| | | Wholemeal bread—Pre-trial | Wholemeal bread 10/11 | White bread 4/13 |
| *Englyst GLC* | | | | |
| NSP: | $\bar{x}$ | 9·57 | 9·33 | 2·78 |
| | *r* | 1·73 | 1·69 | 0·86 |
| | *R* | 3·50 | 3·83 | 1·63 |
| NSP + RS: | $\bar{x}$ | 10·46 | 10·69 | 3·93 |
| | *r* | 1·51 | 1·94 | 0·68 |
| | *R* | 3·27 | 5·40 | 2·15 |
| RS: | $\bar{x}$ | 0·85 | 0·91 | 1·14 |
| | *r* | 0·74 | 0·87 | 0·35 |
| | *R* | 1·10 | 1·00 | 0·51 |
| *Englyst Colorimetric* | | | | |
| NSP: | $\bar{x}$ | 10·59 | 10·66 | 3·08 |
| | *r* | 1·29 | 1·79 | 1·55 |
| | *R* | 3·00 | 5·23 | 1·79 |
| NSP + RS: | $\bar{x}$ | 11·48 | 11·45 | 4·19 |
| | *r* | 1·20 | 2·11 | 1·78 |
| | *R* | 2·69 | 5·26 | 2·04 |
| RS: | $\bar{x}$ | 0·90 | 0·91 | 1·04 |
| | *r* | 0·91 | 2·02 | 1·11 |
| | *R* | 1·42 | 2·09 | 1·36 |
| *FMBRA:* | | | | |
| Dietary Fibre: | $\bar{x}$ | 10·51 | 8·77 | 2·24 |
| | *r* | 1·55 | 1·78 | 2·16 |
| | *R* | 3·05 | 4·63 | 2·73 |

*Key*

$\bar{x}$: mean of results (from Tables I–XIX).
*r*: repeatability of results (from Tables I–XIX).
*R*: reproducibility of results (from Tables I–XIX).

4.4 *Sodium Acetate Buffer, 0·1 mol/l, pH 5·2*

Dissolve 13·6 g sodium acetate trihydrate, $CH_3COONa.3H_2O$, in water and make up to 1 litre with benzoic acid solution (4.3). Adjust to pH 5·2 with acetic acid, 0·1 mol/l. To stabilize and activate enzymes, add 4 ml of calcium chloride solution, 1 mol/l, to 1 litre of buffer.

4.5 *α-Amylase Enzyme:*

α-Amylase, EC 3.2.1.1.; Pancrex V Capsules (approximately 9000 BP units α-amylase per capsule (Paines and Byrne 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 minutes and use the supernatant liquid as α-amylase solution. Prepare immediately before use.

TABLE XXII

DIETARY FIBRE COLLABORATIVE TRIAL SUMMARY OF MEANS, REPEATABILITIES AND REPRODUCIBILITIES FOR THREE METHODS

| Method | | Sample | | |
|---|---|---|---|---|
| | | 1 : 1 Mix white and wholemeal breads 5/9 | High bran bread 2/14 | Rye bread 6/8 |
| *Englyst GLC* | | | | |
| NSP: | $\bar{x}$ | 6·66 | 10·45 | 4·10 |
| | *r* | 2·20 | 3·08 | 1·15 |
| | *R* | 3·01 | 3·75 | 2·30 |
| NSP + RS: | $\bar{x}$ | 7·75 | 10·98 | 5·53 |
| | *r* | 2·97 | 3·96 | 0·63 |
| | *R* | 3·95 | 5·40 | 2·82 |
| RS: | $\bar{x}$ | 0·96 | 0·62 | 1·30 |
| | *r* | 1·02 | 0·70 | 0·79 |
| | *R* | 1·34 | 0·79 | 1·08 |
| *Englyst Colorimetric* | | | | |
| NSP: | $\bar{x}$ | 7·43 | 11·53 | 4·71 |
| | *r* | 2·73 | 1·54 | 1·75 |
| | *R* | 3·39 | 4·40 | 2·02 |
| NSP + RS: | $\bar{x}$ | 8·53 | 11·85 | 6·08 |
| | *r* | 2·87 | 4·60 | 0·97 |
| | *R* | 3·15 | 5·70 | 2·10 |
| RS: | $\bar{x}$ | 0·97 | 0·85 | 1·10 |
| | *r* | 1·63 | 1·99 | 0·67 |
| | *R* | 1·60 | 2·38 | 1·52 |
| *FMBRA* | | | | |
| Dietary Fibre: | $\bar{x}$ | 6·55 | 10·58 | 3·75 |
| | *r* | 2·53 | 3·13 | 1·46 |
| | *R* | 3·93 | 5·41 | 3·28 |

*Key*
$\bar{x}$: mean of results (from Tables I–XIX).
*r*: repeatability of results (from Tables I–XIX).
*R*: reproducibility of results (from Tables I–XIX).

4.6 *Pullulanase Enzyme*

Pullulanase, EC 3.2.1.41 (Boehringer 108944), 100 units/ml.

4.6.1 *Pullulanase Solution, 1 unit/ml*

Dilute pullulanase (4.6) 1 : 100 (e.g. 0·010 ml made to a final volume of 1 ml) with acetate buffer (4.4). Prepare immediately before use.

4.7 *Ethanol, Absolute*

4.8 *Ethanol, 85 per cent. v/v*

4.9 *Sulphuric Acid, 12 mol/l*

### 4.10 *GLC Internal Standard Solution, 1 mg/ml*

Accurately weigh allose (dried to constant weight under reduced pressure with $P_2O_5$) and make up the volume with benzoic acid solution (4.3).

### 4.11 *Octan-2-ol*

### 4.12 *Ammonium Hydroxide, 12 mol/l*

TABLE XXIII

DIETARY FIBRE COLLABORATIVE TRIAL SUMMARY OF MEANS, REPEATABILITIES AND REPRODUCIBILITIES FOR THREE METHODS

| Method | | Sample | |
|---|---|---|---|
| | | Cornflakes 1/7 | Oat Cereal 3/16 |
| *Englyst GLC* | | | |
| NSP: | $\bar{x}$ | 0·81 | 8·23 |
| | *r* | 0·65 | 2·04 |
| | *R* | 1·16 | 2·67 |
| NSP + RS: | $\bar{x}$ | 4·18 | 8·45 |
| | *r* | 2·02 | 4·53 |
| | *R* | 2·46 | 5·12 |
| RS: | $\bar{x}$ | 3·25 | 0·17 |
| | *r* | 1·81 | 0·14 |
| | *R* | 2·32 | 0·70 |
| *Englyst Colorimetric* | | | |
| NSP: | $\bar{x}$ | 1·09 | 8·73 |
| | *r* | 0·91 | 2·13 |
| | *R* | 1·14 | 4·43 |
| NSP + RS: | $\bar{x}$ | 3·89 | 9·30 |
| | *r* | 1·72 | 1·36 |
| | *R* | 1·97 | 4·93 |
| RS: | $\bar{x}$ | 2·87 | 0·36 |
| | *r* | 1·43 | 0·59 |
| | *R* | 1·95 | 1·06 |
| *FMBRA* | | | |
| Dietary Fibre: | $\bar{x}$ | 0·01 | 2·96 |
| | *r* | 0·00 | 1·77 |
| | *R* | 0·07 | 2·40 |

*Key*
$\bar{x}$: mean of results (from Tables I–XIX).
*r*: repeatability of results (from Tables I–XIX).
*R*: reproducibility of results (from Tables I–XIX).

### 4.13 *Ammonium Hydroxide/Sodium Borohydride Solution*

A solution of ammonium hydroxide, 3 mol/l, containing 50 mg sodium borohydride ($NaBH_4$) per ml.

### 4.14 *1-Methylimidazole*

### 4.15 *Acetic Acid, Glacial*

### 4.16 *Acetic Anhydride*

### 4.17 *Potassium Hydroxide, 7·5 mol/l*

4.18 *Sodium Chloride/Boric Acid Solution*

Dissolve 2 g of sodium chloride, NaCl, and 3 g of boric acid, $H_3BO_3$, in 100 ml of water.

4.19 *Sulphuric Acid, Concentrated*

4.20 *Dimethylphenol Solution*

Dissolve 0·1 g of 3,5 dimethylphenol $(CH_3)_2C_6H_3OH$, in 100 ml of glacial acetic acid (4.15).

4.21 *Glucuronic Acid (solid)*

Solid (dried to constant weight under reduced pressure with $P_2O_5$), for preparation of standards used in uronic acid determination.

4.22 *Standard Sugar Solutions, 1 mg/ml*

Use pure preparations of L-rhamnose, L-arabinose, D-xylose, D-mannose, D-galactose and D-glucose (dried to constant weight under reduced pressure with $P_2O_5$). Dissolve the sugars in, and make up the volume with, benzoic acid solution (4.3).

4.23 *Sulphuric Acid, 2 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 *Magnetic Stirrer Hot Plate*

Fitted with a beaker of water 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*

5.7 *Spectrophotometer*

5.8 *Oven*

5.9 *GLC Chromatograph*

GLC Chromatograph fitted with flame ionization detector and preferably, peak area integrator and auto injector.

5.10 *GLC Column and Conditions*

Column: 2·1 m × 2 mm i.d. glass column packed with Supelcoport (100/120 mesh) coated with 3 per cent. SP 2330.

Conditions must be selected to separate and quantitate alditol acetate derivatives of the individual sugars. The method described has been developed based on the following conditions; but others are available.

Injector temperature : 275°C
Column temperature : 215°C (isothermal)
Detector temperature : 275°C
Carrier gas : Nitrogen
Carrier gas flow-rate : 25 ml/min

A typical chromatogram is given in Figure 1. When tailing from the solvent front increases repack the first 5 cm of the column and recondition.

## 6. PROCEDURE

### 6.1 *Pre-treatment of sample*

Foods are analysed without pre-treatment 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 homogenized 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 each sample, each portion weighing between 100 and 500 mg (containing not more than 200 mg of dry matter and 50 mg of NSP, e.g. 200 mg of flour or 100 mg of bran), into 50 ml screw-top centrifuge tubes (5.2). Add a stirrer (5.4) to each. Portion (a) will be used to measure the total NSP content of the sample; portion (b) will be used to measure total NSP together with its resistant starch content.

### 6.3 *Fat Extraction*

Dry samples (i.e. 90–100 per cent. dry matter) with less than 5 per cent. of fat may be analysed directly. Otherwise add 40 ml of acetone (4.1), mix for 30 minutes using a magnetic stirrer, centrifuge and remove by aspiration as much of the supernatant liquid as possible without disturbing the residue. Dry it with stirring at 62·5 ± 2·5°C (see 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) containing test portion (a), and mix for about 2 min at room temperature, using the magnetic stirrer. Add 10 ml of acetate buffer (4.4) to tube (b) containing test portion (b). Cap and place tubes (a) and (b) into the beaker with boiling water (5.3) for 1–1·25 h, 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), pre-equilibrated at 50 ± 5°C, and vortex mix. Then remove tube (b) from the beaker.

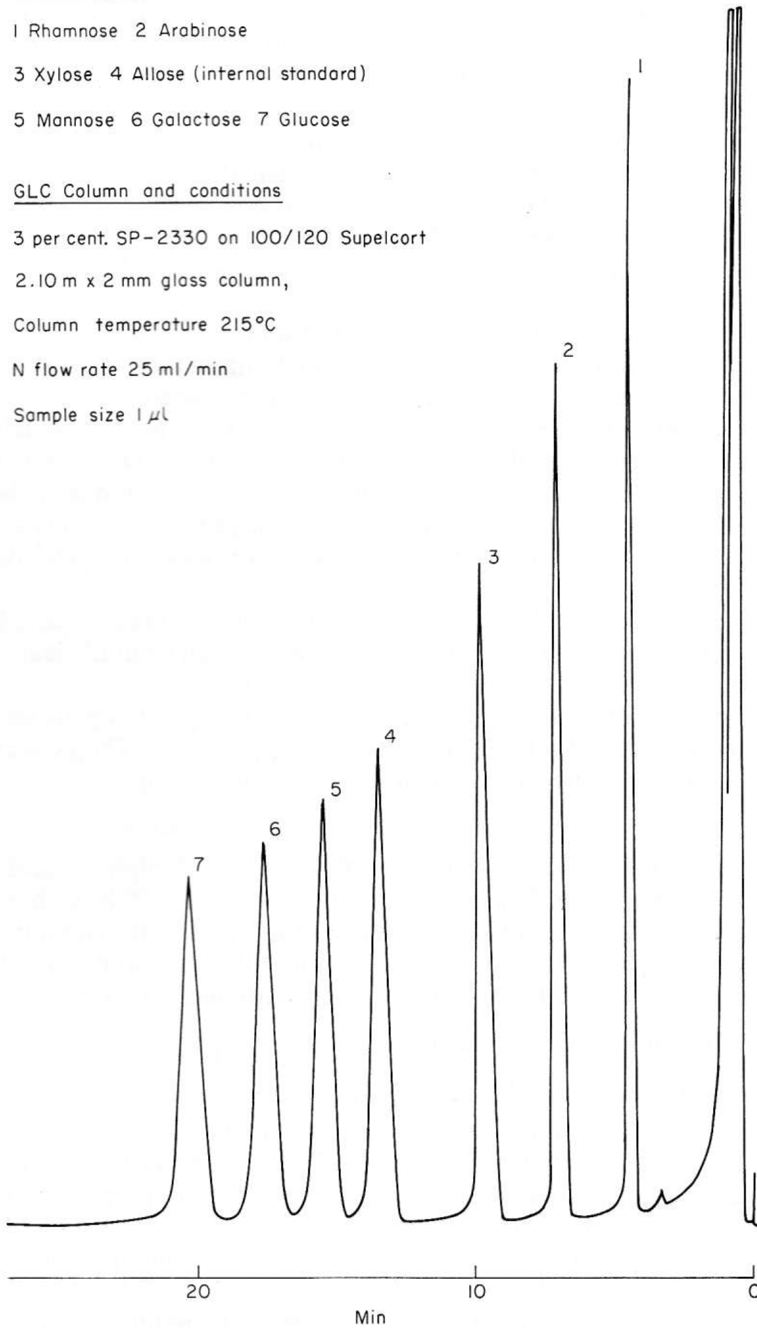


Fig. 1. Typical chromatogram of standard sugars.

### 6.4.2 *Enzymic Hydrolysis of the Starch*

Note: all portions of the samples in their associated tubes are to be treated in the same manner in the procedure given in Section 6.4.2 through to Section 6.7 of the method. Reference to "a sample" or "a tube" is to be taken to include "all samples" or "all tubes" being analysed in this part of the method.

Allow to cool, either at room temperature or in a water bath at 35°C to between 30°C and 40°C, and then immediately add 0·5 ml of α-amylase solution (4.5.1) followed by 0·1 ml of pullulanase solution (4.6.1) and vortex mix. (N.B. Do not mix the enzyme solutions before addition.)

Incubate the sample at 42 ± 2°C (5.6 or 5.8) for 16–18 h. 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 h at room temperature. Centrifuge at 1500 **g** for 10 min or until a clear supernatant liquid is obtained.

Without disturbing the residue, remove by aspiration as much of the supernatant liquid as possible and discard it. Wash the residue twice with 85 per cent. 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 5–10 min), centrifuge until clear and remove the supernatant liquid by aspiration and discard as before.

Add 40 ml of acetone (4.1) to the washed residue, stir magnetically for 5 min and then centrifuge at 1500 **g** for approximately 10 min or until clear. Remove the supernatant liquid by aspiration and discard it.

Place the tube in a beaker of water at 62·5 ± 2·5°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 ± 1°C (5.6) for 1–1·25 h, with occasional mixing, to disperse the cellulose. Rapidly add 22 ml of water and mix. Place in boiling water (5.3) for 2–2·25 h from re-boiling, stirring continuously. Cool and add 5 ml of GLC internal standard solution (4.10) and vortex mix.

### 6.6 *GLC Determination of Neutral Sugars*

### 6.6.1 *Preparation of Alditol Acetate Derivatives*

Add 0·2 ml of ammonium hydroxide, 12 mol/l (4.12) to 1 ml of each hydrolysate (6.5). Test that the solution is alkaline, add a little more ammonium hydroxide if required, and then add 0·1 ml of a freshly prepared ammonium hydroxide/sodium borohydride solution (4.13) and 1–5 μl of octan-2-ol (4.11). Mix, leave for 1 h at 40°C, in a hot block (5.3.1) add 0·1 ml of glacial acetic acid (4.15) and mix again.

To 0·5 ml of the acidified solution add 0·5 ml of 1-methylimidazole (4.14), 5 ml of acetic anhydride (4.16) and mix. Leave for 10 min and then add 0·6 ml of ethanol (4.7) and mix. After 5 min add 5 ml of water, mix and leave for a further 5 min. Place the tubes in a water-bath at room temperature. Add 5 ml of KOH,

7·5 mol/l (4.17) and a few minutes later a further 5 ml of KOH, 7·5 mol/l (4.17). Cap each tube and mix by repeated inversion. Leave the tubes for 10 min or until the separation into two phases is complete. Draw the top phase into a Pasteur or automatic pipette.

If any of the lower phase is included, allow this to separate then run it out before transferring the top phase alone to a small vial. Store at 5°C; the solution is stable for 1–2 weeks.

Mix each of the standard sugar solutions (4.22) with an equal volume of internal standard (4.10). Then dilute 1 : 1 v/v with sulphuric acid, 2 mol/l (4.23). Carry these mixtures through the procedure (6.6.1) with the sample hydrolysates.

6.6.2 *GLC Determination*

6.6.2.1 Carry out conventional GLC determination of the alditol acetate derivatives obtained from section 6.6.1 using the GLC column and conditions given in section 5.9 and 5.10; use 1–2 μl for injection on the chromatograph.

6.7 *Uronic Acids*

Mix 0·3 ml of the aqueous dispersion obtained from section 6.5 (diluted if necessary to contain no more than 100 μg of uronic acids per ml: e.g. no dilution for flour, 1 : 5 for bran) with 0·3 ml of sodium chloride/boric acid mixture (4.18) in 40–50 ml tubes. Add 5 ml of concentrated sulphuric acid (4.19) and vortex mix. Place the tubes in a heating block (5.3.1) at 70°C. Leave for 40 ± 1 min and cool to room temperature by placing in water.

When cool, add 0·2 ml of dimethylphenol solution (4.20) and vortex mix immediately. Between 10 and 15 min later, read the absorbance at 400 and 450 nm in a spectrophotometer (5.7) against a water reference.

Subtract the reading at 400 nm from that at 450 nm for each test sample. Plot the difference in absorbance obtained for glucuronic acid (4.21) standards over the range 25–125 μg/ml. Calculate or read sample concentrations from the graph.

7. EXPRESSION OF RESULTS

7.1 *Calculation of Neutral Sugars*

Calculate the amount of each sugar in g/100 g by the following formula:

$$\frac{W_s \times A_t \times 100}{A_s \times W_t} = \text{per cent. sugar}$$

where

$W_s$ is the weight of internal standard in mg.
$A_t$ and $A_s$ are the peak areas for the test solution and the internal standard, respectively.
$W_t$ is the weight (in mg) of test sample taken for analysis. When using an auto-injector and a computing integrator, the internal standard (4.10) may be

added as a constant percentage (e.g. 5) of the sample weight, thus allowing all calculations by computer as follows:

$$\frac{A_t \times P_s}{A_s} = \text{per cent. sugar}$$

where $P_s$ is the internal standard as weight percentage of the sample taken for analysis; i.e. $P_s = (W_s/W_t) \times 100$.

7.2 *Corrections*

Experiments with mono- and polysaccharides have shown that the hydrolysis and derivatisation procedures result in losses of approximately 10 per cent. of sugars. In order to express results as polymers or anhydro sugars, the GLC results should be decreased by approximately 10 per cent. Because these two corrections are of approximately the same value, however, the GLC results are calculated and expressed directly as polymers or anhydro sugars.

There is incomplete hydrolysis and acetylation (50 per cent.) of any rhamnose. This is corrected for by applying a factor of ×2 to the rhamnose values as determined experimentally. In practice, only traces of rhamnose are present in food products.

7.3 *Total Non-starch Polysaccharides (NSP)*

Total NSP = rhamnose × 2 + arabinose + xylose + mannose + galactose + glucose, all as measured in section 7.1 for test portion (a) + uronic acids as measured in section 6.7 for test portion (a).

7.3.1 *Total Non-starch Polysaccharides, together with Resistant Starch (RS)*

Total NSP + RS = Rhamnose × 2 + arabinose + xylose + mannose + galactose + glucose all as measured in section 7.1 for test portion (b) + uronic acids as measured in section 6.7 for test portion (b).

7.3.2 *Resistant Starch (RS)*

RS = glucose measured for test portion (b), section 7.3.1 minus glucose measured for test portion (a), section 7.3.

All values above are expressed as grams of polymers in 100 g of material taken for analysis.

## APPENDIX II

### ENGLYST PROCEDURE FOR DETERMINATION OF DIETARY FIBRE AS NON-STARCH POLYSACCHARIDES: MEASUREMENT OF CONSTITUTED SUGARS BY COLORIMETRY

### 1. SCOPE AND FIELD OF APPLICATION

The method determines dietary fibre as non-starch polysaccharides (NSP).

### 2. DEFINITION

The content of dietary fibre: the content of non-starch polysaccharides 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 resistant starch content if required.

## 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 per cent. saturated solution*

Dilute saturated benzoic acid solution 1 : 1 v/v with water.

4.4 *Sodium Acetate Buffer, 0·1 mol/l, pH 5·2*

Dissolve 13·6 g sodium acetate trihydrate, $CH_3COONa.\ 3H_2O$, and make up to 1 litre with benzoic acid solution (4.3). Adjust to pH 5·2 with acetic acid, 0·1 mol/l. To stabilise and activate enzymes, add 4 ml of calcium chloride solution, 1 mol/l to 1 litre of buffer.

4.5 *α-Amylase Enzyme*

α-Amylase EC 3.2.1.1; Pancrex V Capsules (approximately 9000 BP units α-amylase per capsule, Paines and Byrne 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 Enzyme*

Pullulanase, EC 3.2.1.41 (Boehringer 108944), 100 units/ml.

4.6.1 *Pullulanase Solution, 1 unit/m*

Dilute pullulanase (4.6) 1 : 100 (e.g. 0·010 ml made to a final volume of 1 ml) with acetate buffer (4.4.4). Prepare immediately before use.

4.7 *Ethanol, Absolute*

4.8 *Ethanol, 85 per cent. v/v*

4.9 *Sulphuric Acid, 12 mol/l*

4.10 *Glucose Solution, 0·5 mg/ml*

Dissolve 25 mg of glucose in 50 ml of saturated benzoic acid solution (4.3).

4.11 *Sodium Hydroxide*

4.12 *Sodium Hydroxide Solution, 3·9 mol/l*

### 4.13 *Sodium-Potassium Tartrate*

### 4.14 *Dinitrosalicylate Solution*

Dissolve 10 g of 3,5-dinitrosalicylic acid, 16 g of sodium hydroxide (4.11) and 300 g of sodium-potassium tartrate (4.13) in redistilled 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.

### 4.15 *Standard Sugar Solutions*

Dissolve 600 mg of arabinose, 800 mg of xylose and 600 mg of glucose in 50 per cent. saturated 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 solution. Add 4 ml of sulphuric acid, 2 mol/l (4.16) to give standards of 0·5, 1·0, 1·5 and 2·0 mg of total sugars/ml in sulphuric acid, 1 mol/l.

### 4.16 *Sulphuric Acid, 2 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 *Magnetic Stirrer Hot Plate*

Fitted with a beaker of water 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 a 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*

### 5.8 *Oven*

## 6. PROCEDURE

### 6.1 *Pre-treatment of sample*

Foods are analysed without pre-treatment 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 each sample, each portion weighing between 100 and 500 mg (but containing not more than 200 mg of dry matter and 50 mg of NSP e.g. 200 mg of flour and 100 mg of bran), into a 50 ml screw-top centrifuge tube (5.2) and add a stirrer (5.4).

Portion (a) is used to measure the total NSP content of the sample; portion (b) is used to measure total NSP together with resistant starch.

6.3 *Fat Extraction*

Dry samples (i.e. 90–100 per cent dry matter) with less than 5 per cent. fat may be analysed directly. Otherwise add 40 ml of 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. Dry it with stirring at 62·5 ±2·5°C (see 6.4.2).

6.4 *Dispersion and Enzymic Hydrolysis*

6.4.1 *Dispersion of Starch*

Add 2 ml of DMSO (4.2) to tube (a) containing test portion (a) and mix for about 2 min at room temperature, using the magnetic stirrer. Add 10 ml of acetate buffer (4.4) to tube (b) containing test portion (b). Cap and place tubes (a) and (b) in the beaker with boiling water (5.3) for 1–1·25 h 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), pre-equilibrated at 50 ±5°C, and vortex mix. Then remove tube (b) from the beaker.

6.4.2 *Enzymic Hydrolysis of the Starch*

Note: all portions of the sample in their associated tubes are to be treated in the same manner in the procedures given in Section 6.4.2 through to Section 6.6 of the method. Reference to "a sample" or "a tube" is to be taken to include "all samples" or "all tubes" being analysed in this part of the method.

Allow the tubes to cool, either at room temperature or in a water bath at 35°C, to between 30 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 ±2°C (5.6 or 5.8) for 16–18 h. 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 h 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 liquor as possible (without disturbing the residue) and discard it. Wash the residue twice with 85 per cent.

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 5–10 min), centrifuge until clear and remove the supernatant liquor by aspiration as before.)

Add 40 ml of acetone (4.1) to the washed residue, stir for 5 min and then centrifuge at 1500 **g** for approximately 10 min or until clear. Remove the supernatant liquor by aspiration and discard it. Place the tube in a beaker of water at 62·5 ±2·5°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 ±1°C (5.6) for 1–1·25 h, with occasional mixing, to disperse the cellulose. Rapidly add 22 ml of water and mix. Place in boiling water (5.3) for 2–2·25 h from re-boiling, stirring continuously. Cool to room temperature.

### 6.6 *Measurement of Total Reducing Sugars*

Place into separate test tubes 1 ml of blank solution (1 : 1 v/v mixture of 50 per cent. saturated benzoic acid and sulphuric acid, 2 mol/l), 1 ml of each of the standard solutions (4.15) and 1 ml of the hydrolysate (6.5). Add 0·5 ml of glucose solution, 0·5 mg/ml (4.10), and 0·5 ml of sodium hydroxide, 3·9 mol/l, (4.12) 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. EXPRESSION OF RESULTS

### 7.1 *Calculation*

#### 7.1.1. *Calculation of Non-starch Polysaccharides*

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

$$\frac{A_t \times V_t \times 100}{A_s \times W_t}$$

where $A_t$ is the absorbance of the test solution obtained from tube (a), $V_t$ is the total volume 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).

#### 7.1.2 *Calculation of Non-starch Polysaccharides together with Resistant Starch*

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

$$\frac{A_t \times V_t \times 100}{A_s \times W_t}$$

where $A_t$ is the absorbance of the test solution obtained from tube (b), $V_t$ is the total volume 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 standards and $W_t$ is the weight (mg) of sample taken for analysis in tube (b).

## APPENDIX III

## ESTIMATION OF DIETARY FIBRE FROM THE TOTAL PENTOSE CONTENTS OF WHEAT PRODUCTS "RAPID" METHOD FROM THE FLOUR MILLING AND BAKING RESEARCH ASSOCIATION

### 1. SCOPE AND FIELD OF APPLICATION

The method determines total pentose in wheat products, from which an estimate of the content of dietary fibre (DF), including lignin, is derived.

### 2. DEFINITION

Estimate of dietary fibre: the value for DF from a predetermined correlation of DF with total pentose, where total pentose is determined by the method specified, and DF is determined by the Englyst method, with separate analysis for lignin.

### 3. PRINCIPLE

The sample is partially hydrolysed with dilute sulphuric acid to bring pentosans into solution. After centrifuging, an aliquot of diluted supernatant is taken for determination of total pentose, using a colorimetric procedure. Pentose is expressed as xylose.

### 4. REAGENTS

High purity reagents and distilled or deionised water are used throughout the method.

4.1 *Sulphuric acid 0·5 mol/l*

4.2 *Xylose*

Solid, for preparation of standard solution, dried over phosphorus pentoxide under vacuum.

4.2.1 *Xylose, standard solution, 100 μg/ml*

Aqueous solution of 25 mg of xylose made up to 250 ml, using water saturated with benzoic acid as a preservative (stable for up to 6 months at 0–4°C).

4.3 *Glucose solution*

Dissolve 1·75 g glucose in 100 ml water.

4.4 *Hydrochloric acid, concentrated, sp. gr. 1·18*

4.5 *Acetic acid, glacial*

Some brands turn the reagent pink shortly after preparation. Analar acetic acid supplied by BDH Ltd., Poole, is recommended.

4.6 *Phloroglucinol, solid*

Laboratory reagent grade is satisfactory.

4.6.1 *Phloroglucinol reagent*

Prepare fresh by dissolving 1 g of phloroglucinol (4.6) in 5 ml 95 per cent. ethanol by warming. Add 110 ml of acetic acid (4.5), 1 ml of 1·75 per cent. glucose solution (4.3) and 2 ml of hydrochloric acid (4.4) immediately prior to use.

5. APPARATUS

5.1 *Magnetic stirrer hotplate*

A hotplate capable of boiling a water bath large enough to contain up to 16 tubes supported in a rack. The magnetic torque should be sufficient to rotate the stirrers (5.2) in all the tubes.

5.2 *Magnetic stirrers*

PTFE-coated, to fit centrifuge tubes 5.3.

5.3 *Centrifuge tubes*

Screw-capped glass centrifuge tubes of 25–30 ml capacity are ideal, but stoppered tubes (15–50 ml) may be used. To prevent evaporation during boiling, stoppers should be held in place by elastic bands.

5.4 *Centrifuge*

Capacity 8 to 16 tubes, capable of approximately 2500 r.p.m. (about 1875 **g**).

5.5 *Volumetric flasks*

Capacity 10 to 250 ml.

5.6 *Glass tubes*

Stoppered tubes of 25 ml capacity for pentose estimation.

5.7 *Pipette*

A 10 ml bulb pipette attached to a syringe, for dispensing acetic acid-containing reagent 4.6.1. A plastic disposable syringe may be used.

5.8 *Spectrophotometer/colorimeter*

A narrow bandpass instrument for determining absorbances at 552 and 510 nm.

5.9 *Cuvettes*

Cells of 10 mm path length or 10 mm diameter tubes.

6. PROCEDURE

6.1 *Pre-treatment of sample*

Extraction of fat (see 6.4) and/or drying may be required. Coarse samples are ground to pass a 1 mm gauze.

6.2 *Bulk sampling*

Take two separate bulk samples, where possible.

6.3 *Analytical samples*

From each bulk sample accurately weigh to nearest 0·1 mg a sub-sample to contain 200 ± 20 mg of dry matter, after fat extraction if necessary.

6.4 *Fat extraction*

May be required with high fat samples. Add 20 ml of acetone, mix for 30 min using a magnetic stirrer, centrifuge, and remove by aspiration as much of the supernatant liquor as possible without disturbing the residue. Air-dry the residue, or dry it by mixing at 60–62°C until no acetone remains. It may be possible to avoid fat extraction, if centrifuging (6.6) gives a clear layer of hydrolysate from which the aliquot (6.7) can be taken.

6.5 *Prehydrolysis*

Add 10 ml sulphuric acid of 0·5 mol/l (4.1), using it to wash down particles adhering to the tube walls. Place tubes in boiling water bath with magnetic stirring for 0·5 h. Cool to room temperature in water. Centrifuge at *c.* 2500 r.p.m. (*c.* 1875 **g**) for 10 min.

6.6 *Dilution of hydrolysate*

Dilute an aliquot of the supernatant liquor with water to contain 25–75 μg/ml of pentose and less than 0·1 mol/l sulphuric acid. Typical dilutions are:

| | |
|---|---|
| White flour | 1–10 ml |
| Wholemeal flour | 1–25 ml |
| Offal | 1–50 ml |
| Bran | 1–100 ml |

6.7 *Determination of pentose*

Add 10 ml of phloroglucinol reagent (4.6.1) to a 2 ml aliquot of diluted hydrolysate (6.6) and to 2 ml aliquots of standards containing 0–150 μg pentose, prepared from xylose solution 100 μg/ml (4.2.1).

Place the tubes in a boiling water bath for 25 min. Cool in water for 5 min. Immediately read the absorbances at 552 nm then 510 nm, with water in the reference cell. Tubes should be placed in water bath at 5 min intervals, to eliminate variable delays before reading, as the colour is subject to fairly rapid fading.

NB The reagent should be used for no more than 20–25 samples. If samples and standards to be analysed exceed this number, the analysis should be planned so that replicates are in separate blocks, with fresh reagent for each block.

Subtract the reading at 510 nm from that at 552 nm for each sample. Plot the difference in absorbance obtained from xylose standards against the concentration and calculate sample concentrations using the line of best fit.

## 7. EXPRESSION OF RESULTS

### 7.1 *Calculation of pentose*

The percentage of pentose in the sample is calculated as xylose from the formula:

$$\frac{\text{mg xylose} \times \text{dilution} \times 100}{\text{mg sample}}$$

and adjusted to dry matter basis, using the pre-determined moisture content.

### 7.2 *Estimation of dietary fibre*

The dietary fibre content is calculated from the predetermined calibration equation of DF against total pentose (both on a dry matter basis) for a variety of wheat products: DF Englyst = 1·75 Pentose −0·87.

The DF Englyst value includes lignin, determined separately.

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# Book Reviews

PESTICIDE EFFECTS ON SOIL MICROFLORA. Edited by L. SOMERVILLE and M. P. GREAVES. London: Taylor and Francis, 1987. Price £35.00.

Pesticides are biologically active compounds and must therefore be expected to offer some potential risk, however small, to man and his environment. Most governments exercise control over the supply and use of pesticides through a registration procedure which is essentially predictive. Registration implies the acceptance by a competent authority of evidence submitted in support of a company's claims for the safety and efficacy of the proposed pesticide product and its use.

Over about 30 years, scientists in governments and industry have developed an extensive programme of testing in plants and experimental animals. Data from this programme are extrapolated with reasonable confidence to predict the effects of a pesticide on man and his animals. Unfortunately the "state of the art" in test procedures which can produce data relevant to predicting some environmental effects has not progressed nearly as well. By comparison with the above, guidance on reliable test procedures to estimate the effects of pesticides on soil micro-organisms is almost non-existent, anywhere in the world.

The work described in this book is an attempt to improve the situation. Groups of interested scientists have met and corresponded since 1978 to assess the current meagre guidelines, and to develop recommendations for appropriate test procedures for assessing the effects of pesticides on soil microflora. Official registration scientists, however, were not involved.

The task has clearly been an uphill struggle. Chapter 15 starts by admitting that one of the most important functions of the international workshops has been to identify the limitations of current methods. Many of these limitations have been known for some time and although Annex B uses paragraphs from the FAO Consultation on Environmental Criteria for the Registration of Pesticides, Rome, May 1981, there is no reference anywhere in the book to this basic document on the general requirements for environmental criteria for the registration of pesticides.

The main reason for slow progress is identified in a sentence in Chapter 14 (and misquoted with a wrong reference two pages later)—"There is inadequate knowledge of what a soil ecosystem is and which processes and organisms are of major importance in maintaining soil quality". Natural variability in soil microbial activity is inevitable, and many environmental factors may influence micro organism populations and their activity.

Even if reliable and reproducible methodology is developed, the extrapolation of laboratory studies to the field is unproven, and the ecological significance of any predicted local effect is unknown.

Several chapters summarising experimental data are useful in defining the present state of knowledge but contribute little to identifying new methodology which may be of value to registration authorities. The recommended laboratory tests in Appendix B have already been published separately and made widely available to interested parties.

The transposition of Figures 1 and 2 in the first chapter get the reader off to a somewhat poor start, but the text in general is easy to read, regularly emphasising how little we really do know about soil ecosystems. The reviewer can only fully support the final inevitable recommendation that "more research is essential". But there must be some doubt as to whether a £35.00 book is needed to get this message across.

J. A. R. BATES

ICUMSA PROCEEDINGS 19TH SESSION CANNES 1986. Peterborough: ICUMSA, 1987. Hardback. Price £28.00. 466 pp. + index. ISBN: 0 905003 11 X.

This is the latest in the series of ICUMSA proceedings and covers the meeting of 117 delegates from 28 major sugar-producing countries at Cannes in 1986.

ICUMSA, the International Commission for Uniform Methods of Sugar Analysis addresses itself to the unified approach to sugar analysis and plays a vital role in the drawing up of methodology for use by the trade. There are close links with IUPAC, the AOAC and the APA, and although its purpose is not to formulate statutory methods, it plays a significant part in that work.

This book is best described as a full and frank account of the meetings and it includes not only details of methodology but a verbatim report of all of the discussions. For this reason it does not lend itself to routine laboratory use, since the reader would have difficulty extracting the relevant information quickly. Having said this, however, the series does enable the reader to learn of the latest practices in the sugar trade and to discover the trade's thoughts on analytical matters from simple polarimetry to the more esoteric qualities of sugar and sugar products.

The book deals with 31 subjects in 466 pages, and contains much statistical information on the performance of nearly all well used analytical methods. Some useful comparisons of methods are also included, as are details of microbiological tests for sugar.

The book is welcome in the reviewer's library, but should be read in conjunction with the ICUMSA Sugar Analysis Book (1979) which is more useful to a practising analyst.

Close involvement with ICUMSA does not colour the reviewer's judgement of this series—he only wishes he had been at Cannes last summer!

C. J. SHELTON