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DETERMINATION OF DIETARY FIBRE COLLABORATIVE TRIALS

PART IV

[1]A Comparison of the Englyst and Prosky (AOAC) Procedures for the Determination of Soluble, Insoluble and Total Dietary Fibre.

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Thirty seven laboratories from 11 countries took part in an interlaboratory study to compare the accuracy and precision of the Englyst GLC and colorimetric and Prosky gravimetric methods for determination of dietary fibre in food. Twelve foods were analysed as duplicates for total dietary fibre (TDF), soluble and insoluble dietary fibre. Whilst all methods performed reasonably well for both repeatability and reproducibility, systematic differences were seen in the results. The Prosky method gave values that were, on average, 19% higher than the Englyst values for TDF but 35% lower for soluble fibre. The absolute differences in TDF are probably due to the inclusion in the gravimetric residue of some starch made resistant to pancreatic amylase during food processing and sample handling and of non-carbohydrate materials, of which lignin is one. For soluble fibre the differences are related to the *p*H at which soluble fibre is extracted. The inclusion of non-cell wall material such as starch in the Prosky residue means that these value for fibre are more dependent on food processing and less a measure of the plant-cell wall polysaccharides embodied in the dietary fibre hypothesis. We therefore recommend the Englyst GLC and Englyst colorimetric procedures as suitable for measurement of total, soluble and insoluble dietary fibre for food labelling.

Introduction

In 1987, as a result of a series of collaborative trials organised by the Ministry of Agriculture, Fisheries and Food (MAFF)[1-3], the Englyst enzymic-chemical procedure for dietary fibre measured as NSP, with gas-liquid chromatography (GLC) end-point determination of component monosaccharides, was recommended as the reference method for the UK, with the colorimetric procedure suggested as suitable for quality control[3]. The methods have been issued by MAFF as part of the MAFF Validated Methods for the Analysis of Foodstuffs Bulletins[4,5].

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The need for the present trial arose for three main reasons. Firstly, the previous trials had focused entirely on cereal foods. Whilst the Englyst technique has been applied to a wide range of other foods[6,7] and total dietary fibre values, measured as non-starch polysaccharides (NSP) by this method are now incorporated into the British food tables[8], it was suggested that the applicability of the method to non-cereal foods should be tested in a collaborative trial[3].

Secondly, in 1988 the AOAC recommended as Official First Action a modified version of the Prosky procedure for measurement of total dietary fibre[9]. In the initial MAFF trials an earlier version of the Prosky method was tested. It was shown to give higher values for dietary fibre than the direct chemical methods because the gravimetric residue contained substances other than cell-wall materials, such as starch, tannins and co-precipitated salts. It was therefore decided to use the Englyst technique in subsequent trials. However, the Prosky procedure[9] has been modified to overcome some of its problems and so has been included in the present trial both to test its robustness in the hands of routine laboratories and to compare it with the more detailed Englyst technique.

Thirdly, dietary fibre is not a single substance; it includes a wide range of polysaccharides held together in a form that imparts structural characteristics to the plant cell. Physiological studies have shown that fibre from different plant sources has contrasting effects on health. The range of effects is due to a combination of the chemical and physical properties of dietary fibre[10]. One characteristic of fibre that reflects both these properties is solubility in water. Soluble fibre is associated mainly with cholesterol lowering[11] and changes in the glycaemic response to foods[12]. Insoluble fibre is thought to affect primarily the function of the large bowel[13]. These findings have led nutritional scientists and the food industry to look for ways of measuring soluble and insoluble dietary fibre. Both the Englyst[14] and Prosky procedures[9] are claimed to do this and a formal comparison of the two methods for this purpose was therefore undertaken.

Finally the Englyst method has undergone a number of modifications since 1987, which alone justified a further trial of its utility[15].

Method Developments Since 1987

Englyst procedure

The Englyst method, first described in 1982[16], is a development of that of Southgate[17] and aims to estimate plant-cell wall polysaccharides as non-starch polysaccharides (NSP) in foods. In principle, starch is removed enzymically after solubilisation and NSP measured as the sum of the constituent sugars released by acid hydrolysis. Values may be obtained for the soluble and insoluble fibre. Cellulose may be measured separately and the non-cellulosic polysaccharides are characterised by measurement of the individual monosaccharides. The original Englyst used gas chromatography but more recently a rapid colorimetric

modification has been described[18]. The method measures the plant cell wall polysaccharides and chemically related (non-α-glucan) polysaccharides.

In the procedure used in the trial reported in 1987, samples were incubated for 16 hours with pancreatin and pullulanase[3]. The modified procedure used in the present trial includes faster hydrolysis of starch and NSP, thus reducing the overall analysis time for the measurement of total, soluble and insoluble NSP[15]. The combination of Termamyl (Novo), pancreatin and pullulanase results in complete removal of starch after only 50 min incubation. The hydrolysis of NSP has been optimised using 12 mol/l and 2 mol/l sulphuric acid to give complete release and desulphation of constituent sugars in two hours, which was previously obtained in three hours. In Table I it is shown that the recovery is virtually identical after the two sets of hydrolysis conditions.

TABLE I

	Primary Hydrolysis			Secondary Hydrolysis			Recovery (g(100 g)$^{-1}$ sample)					
Reference	(M)	(h)	(°C)	(M)	(h)	(°C)	Ara	Xyl	Man	Gal	GLC	Total
A Englyst	12	1	35	1	2	100	13.9	23.6	0.4	1.1	16.1	55.1
B Englyst	12	1	35	2	1	100	14.6	25	0.5	1.2	17.9	59.2
A Englyst	-	-	-	1	2	100	14.6	24.4	t	1.1	1.2	41.4
B Englyst	-	-	-	2	1	100	14.9	24.8	0.2	1.3	1.4	42.6

Comparison of values obtained for wheat bran subjected to:

A, the conditions previously used in the Englyst procedure[3] (ref.); and

B, the conditions now employed in the Englyst procedure.

Prosky procedure

This method was described first in 1984 by Prosky et al.[19]. The aim of the method was that it should be rapid and measure indigestible carbohydrate as "the sum of the soluble and insoluble polysaccharides and lignin". In principle the method involves gelatinising dried foods with a heat-stable α-amylase and then enzymically digesting them with protease and amyloglucosidase to remove protein and starch. Ethanol is added to precipitate soluble dietary fibre and the residue is filtered, washed and dried. The residue is analysed for protein (N × 6.25) and ash, and dietary fibre is determined as the weight of the residue less protein and ash. The method has been subject to a number of modifications since its inception and to a series of interlaboratory trials[9,20,21]. The gravimetric residue contains principally plant cell-wall polysaccharides, some resistant starch, and non-carbohydrate material including lignin.

The method employed in this trial was supplied directly by Prosky as the latest version available at the time of the trial[22].

Collaborative Trial Organisation

Methods

The methods used in the trial are described in Appendices I - III. They are as follows:

Appendix I: Rapid determination of total, soluble and insoluble dietary fibre by the Englyst procedure: measurement of the constituent sugars by colorimetry.

Appendix II: Determination of total, soluble and insoluble dietary fibre by the Englyst procedure: measurement of constituent sugars by gas-liquid chromatography.

Appendix III: Prosky procedure for the determination of insoluble and soluble dietary fibre in foods (enzymatic - gravimetric method)

Participants

Thirty-seven laboratories participated in the trial, of which 36 returned results; 20 were from the UK and 16 non UK (Belgium, Canada (2), Denmark (3), France, Germany, Greece, Ireland, Italy (2), Japan (2), The Netherlands (2) , South Africa and USA).

Samples

All the samples were commercially prepared, ground and homogenised before dispatch to participants. Because of the nature of the samples, and because a wide range of foodstuffs was to be included in the trial, it was not possible to disguise some of the individual samples to be blind duplicates; these samples were therefore analysed as known duplicates. However, a number of the samples were analysed as blind duplicates where such disguise was practical.

The samples included in the trial are given below:

Sample Type	Sample Code	Sample Type	Sample Code
Coconut	A	Apple	G
Porridge	B	Bread	H
Baked Beans	C	White Bread	I/P
Cabbage	D	Cornflakes	J/K
Mashed Potato	E	Wholemeal Bread	L/M
Banana	F	1:1 Bread Mix	N/O

Results

Results obtained for all methods are given in Tables II to XXXVIII. All results are expressed as g/100g on a dry mater basis throughout Report.

The results are summarised in Tables XXXIX and XXXX.

Statistical Analysis of the Results

The results were first examined for evidence of individual systematic error using Cochran's and Grubb's tests at $P<0.5$ and then statistically analysed by procedures described in the IUPAC/ISO/AOAC Protocol for the Design, Conduct and Interpretation of Collaborative Studies[23].

Calculations for repeatability (r) and reproducibility (R), as defined by the procedures given in that protocol were carried out on those results remaining after removal of outliers. The results for each method investigated and accompanying statistical data are given in Tables II - XXXVIII and summarised in Tables XXXIX and XXXX.

In calculating the precision of the methods, standards for outlier tests were adopted that differ from those used in the previous three trials. Outliers, or excluded values, identified by the Cochran's or Grubb's' tests have been excluded only if they were significantly different at the $p<0.01$ level whereas previously $p<0.05$ was used. The net effect of this change is that there are fewer exclusions and therefore repeatability (r) and reproducibility (R) values will tend to be higher than with trials where outliers are excluded at $p<0.05$.

For all methods less than 5% of results were excluded.

Results and Discussion

Comparison of Mean Values for the Different Methods

A comparison has been made of the mean values for total and soluble dietary fibre by the methods used in this trial. These comparisons are shown diagrammatically in the figures 1 to 4; individual aspects of these figures are described subsequently. In each of the figures the line of unity is shown so that the comparison of mean values for the different combinations of method may be readily appreciated.

The figures are:

Figure 1: A comparison of the mean values for total dietary fibre by the Englyst-GLC and the Englyst colorimetric methods.

Figure 2: A comparison of the mean values for total dietary fibre by Englyst-GLC method and the Prosky methods.

Figure 3: A comparison of the mean values for soluble dietary fibre by the Englyst-GLC and Englyst colorimetric methods.

Figure 4: A comparison of the mean values for soluble dietary fibre by Englyst-GLC method and the Prosky method.

Total dietary fibre

Although the results from the three methods are highly correlated systematic differences occur, particularly between the Englyst-GLC results and those of the Prosky technique.

Englyst GLC and Englyst Colorimetric Methods

For total dietary fibre the Englyst colorimetric method gives slightly higher values than by GLC (average 8.57g GLC versus 8.99g colorimetry; difference 4.9%, t 2.82, $p>0.017$) (see Figure 1 and Table XXXX). However participants in the collaborative trial, because the nature of the samples were not disclosed, were not able to apply the appropriate standard correction factors of × 0.7 for cereals and × 1.03 for vegetables, as used during routine analysis. In practice, the GLC and colorimetric methods give very similar results[16].

Englyst GLC and Prosky Methods

Larger differences for total dietary fibre occur between the Prosky method and the Englyst GLC technique (see Figure 2 and Table XXXX), with the Prosky method consistently giving higher values (10.20g Prosky [mean of all results] versus 8.57g Englyst GLC [mean of all results], difference 19%, t 3.79, $p>0.003$). When the Prosky and the Englyst GLC results are compared for individual foods (Table XXXIX) the differences show great variability and cannot be ascribed to a single cause. The ratio between the two methods varies from near perfect agreement for porridge to a difference of × 2.3 for cornflakes. The differences are mostly for starchy foods suggesting that a major factor explaining them is the inclusion of some starch in the Prosky gravimetric residue although the non-carbohydrate residues will also contribute.

Soluble and Insoluble Dietary Fibre

Englyst GLC and Englyst Colorimetric Methods

Figure 3 shows the results for soluble dietary fibre using the two Englyst methods. They give highly correlated values, but some large systematic differences occur. The GLC and colorimetric values agree very well, 3.69g GLC versus 3.70g colorimetric, which are not significantly different.

Englyst GLC and Prosky Methods

However, the Prosky technique, which gives higher values for total dietary fibre, gives only two-thirds of the value for soluble dietary fibre (2.38g Prosky) (Prosky versus GLC t 4.51, $p>0.001$). This is most probably due to differences in the *p*H at which soluble dietary fibre is extracted in the two methods. In the Englyst GLC procedure, the *p*H is 7, whilst it is 4.8 in the Prosky procedure. The recovery of soluble dietary fibre has been shown to depend on *p*H[24].

Comparison of Precision Values for the Different Methods

A significant number of precision values have been calculated from the results obtained in this trial. These are summarised in Table XXXIX.

Individual sample values have not been compared because of the multiplicity of data; however, the values of reproducibility, R, for each method have been plotted in Figures 5 - 7 for soluble, insoluble and total dietary fibre.

The figures are:

Figure 5: A comparison of the reproducibility values obtained by the Englyst-GLC, Englyst colorimetric and Prosky method for total dietary fibre. Results ordered by mean value obtained using the Englyst-GLC method.

Figure 6: A comparison of the reproducibility values obtained by the Englyst-GLC, Englyst colorimetric and Prosky methods for soluble fibre. Results ordered by mean value obtained using the Englyst-GLC method.

Figure 7: A comparison of the reproducibility values obtained by the Englyst-GLC, Englyst colorimetric and Prosky methods for insoluble fibre. Results ordered by mean value obtained using the Englyst-GLC method.

These Figures indicate that in general the Englyst-GLC method is the most reproducible.

Individual repeatability values have not been compared, but a similar pattern is demonstrated as shown in that for reproducibility.

The absolute values of repeatability and reproducibility are greater than would be expected for measurements at the g/100g concentration level. However, this pattern has been shown in a number of previous collaborative trials, and is commented on further below.

Participant Experience in Dietary Fibre Analysis

Another important factor to take into account when comparing r and R with other trials of dietary fibre methods is the wide scope of participants in the present trial. Invitations to join the trial were widely distributed and within the UK it was open to any public analyst, food company or interested party. The resulting 36 laboratories who took part came from 11 countries in four continents (Africa, Asia, Europe and North America) whilst a number of UK public analysts with no special expertise in this area took part. In some trials of dietary fibre methods (9,21) participating laboratories have mostly been skilled in the particular method of analysis being tested. In the present trial many of the laboratories were doing these methods for the first time.

In general, the Englyst GLC procedure performed the best, with the between laboratory variation (R) for total dietary fibre, being the lowest for 9 out of the 12 test samples. The absolute value for R for all methods was relatively large for samples with low contents of total dietary fibre. For soluble dietary fibre, the Prosky method did not perform quite as well as the other methods for 11 out of the 12 samples, with a coefficient of variation of over 50% for coconut, porridge, cornflakes and the bread mixture, a problem noted in earlier trials[9].

The Aims of the Englyst and Prosky Procedures

The original concept of dietary fibre was based on observations made by Burkitt, Trowell and others[25,26], who postulated that eating a diet rich in unrefined plant foods was protective against a range of diseases that were common in Western societies, where much of the 'dietary fibre' was removed by food refining techniques. Trowell's earliest description of dietary fibre was as "the proportion of food which is derived from the cellular walls of plants which is digested very poorly in human beings"[27]. This hypothesis is a very broad one. A number of dietary components such as fat and total energy contribute to the risk of Western diseases. Nevertheless a considerable body of research now shows that the polysaccharides of the plant cell wall are not digested in the upper intestine of man, but are metabolised in the large bowel by the bacteria and thus exert important physiological effects[28,29]. These properties are of direct importance to public health in the management of diabetes, lowering of blood cholesterol and prevention of bowel diseases especially constipation. They justify consumer interest in fibre and thus in labelling.

It is now clear that the component of the diet for which the term 'dietary fibre' was coined is largely plant cell-wall material, and that the several physiological effects of eating this material depend on both its identity and its physical structure. This does not lead easily to a definition that will allow direct quantification by analysis. However, recognition that the plant cell-wall material in human foods can be defined chemically as non-starch polysaccharides offers a solution. Plant foods contain a wide variety of polysaccharides (polymers of sugars joined by glycosidic linkages), which may be separated into two groups on the basis of both structure and function. The starches are storage polysaccharides and are chemically identifiable as polymers of glucose with α-glucosidic linkages. The remainder, which contain no α-glucosidic linkages, are mostly structural components of the plant cell walls, and may be referred to conveniently as the non-starch polysaccharides (NSP). Dietary fibre measured as NSP provides values that makes them the most useful for food labelling, because they are a marker of the type of diet advocated in dietary guidelines and thus provide the consumer with valuable information. NSP values are unaffected by food processing and are therefore suitable for the construction of food tables, because values for raw foods can be used for the calculation of intakes of dietary fibre from foods and from diets. NSP values are obtained by chemical analysis of a defined group of carbohydrates and are therefore suitable for regulatory purposes.

None of the approaches to measuring dietary fibre, of which the Englyst and Prosky procedures are the most widely known, aims to measure cell-wall material, which would be an almost impossible task. Whilst the Englyst method measures the cell-wall and related polysaccharides, the Prosky procedure includes also some starch and non-carbohydrate material, of which lignin is one component. These differences in approach are exemplified in the results of the present trial. For most foods the Prosky method gives higher values. We have argued in earlier

papers[1,2,3,34] that neither lignin nor any type of starch should be included as part of dietary fibre for food labelling purposes. Resistant starch is not a cell-wall constituent and, as currently defined[34,35], is not measured by the Prosky method, which recovers only part of the starch that resists digestion in man, principally that formed during food processing. Lignin is not a carbohydrate and, although it is part of the plant cell wall and retards the fermentation of NSP in animal studies, it has not been shown to have clear physiological effects in man.

Choice of Methods for Measuring Dietary Fibre

Both the Englyst and Prosky methods can be performed in normally equipped laboratories and gave reasonably reproducible results in this study. Both have been applied to a wide range of foodstuffs. The major difference lies in what is measured. The inclusion of starch and other non-carbohydrate materials that are not part of the plant cell-wall makes the Prosky method a less precise index of dietary fibre than the Englyst method, which specifically aims to isolate cell-wall polysaccharides. Other advantages of the Englyst (GLC) method are the capacity to give detailed information on the amounts of cellulose and non-cellulosic polysaccharides and composition of individual monosaccharides thus allowing some characterisation of the type of fibre, a particularly useful feature for enforcement of labelling claims. The Englyst method is not affected by food processing and is applicable therefore to dietary epidemiological studies, can be used for food tables and for the isolation of cell-wall polysaccharides from gut contents in physiological studies. The same method therefore can be used across the spectrum of interests from experimental work to food labelling legislation. This is not possible with the Prosky method because of the effect of food processing and sample handling will alter the apparent dietary fibre value due to the inclusion of retrograded starch and non-carbohydrate materials.

Conclusion

The trial shows that the Prosky gravimetric method and both the Englyst GLC and colorimetric technique can be applied in a wide range of laboratories to many different types of food with reasonable precision for measurement of total dietary fibre. However consistently higher values for total dietary fibre are obtained using the Prosky method largely because of the inclusion of some starch and other non-cell wall material in the precipitate.

Soluble fibre was measured with acceptable precision by the Englyst methods, but was less robust with the Prosky technique. Substantial differences in the absolute values were shown for the Prosky versus the other methods in the trial for soluble fibre. These differences probably relate to the use of a lower *p*H for extraction of soluble fibre in the Prosky method.

Overall the Englyst GLC and colorimetric procedures can be recommended as reference methods for the determination of total, soluble and insoluble dietary fibre in food. At present the Prosky technique,

because of the inclusion of some starch and other materials not present in the cell wall, cannot be recommended as a measurement of dietary fibre although it performed acceptably in technical terms for measurement of total dietary fibre.

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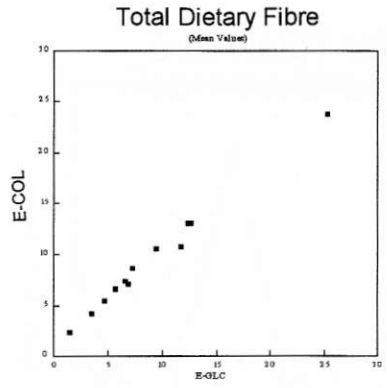


Figure 1:

A comparison of the mean values (g/100g) for total dietary fibre by the Englyst-GLC and Englyst-colorimetric methods.

Figure 2:

A comparison of the mean values (g/100g) for total dietary fibre by the Englyst-GLC and Prosky Methods.

Figure 3:

A comparison of the mean values (g/100g) for soluble dietary fibre by the Englyst-GLC and Englyst-colorimetric methods.

Figure 4:

A comparison of the mean values (g/100g) for soluble dietary fibre by the Englyst-GLC and Prosky methods.

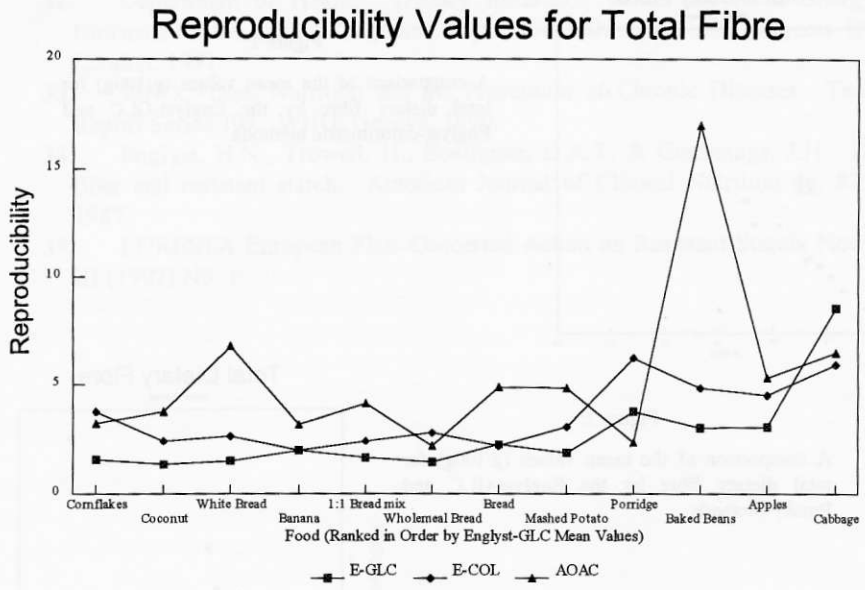


Figure 5:

A comparison of the reproducibility values obtained by the Englyst-GLC, Englyst-colorimetric and Prosky methods for total dietary fibre. Results ordered by mean value obtained using the Englyst-GLC method.

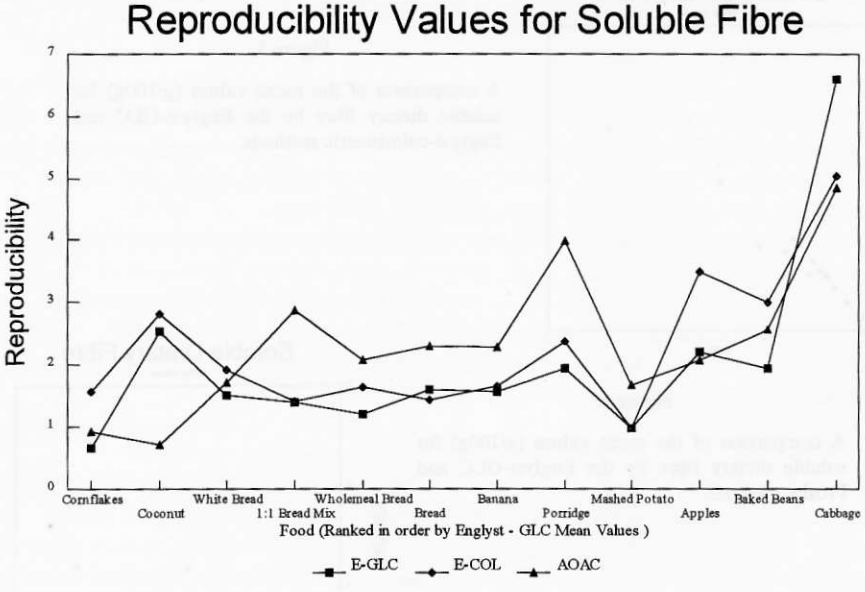


Figure 6:

A comparison of the reproducibility values obtained by the Englyst-GLC, Englyst-colorimetric and Prosky methods for soluble dietary fibre. Results ordered by mean value obtained using the Englyst-GLC method.

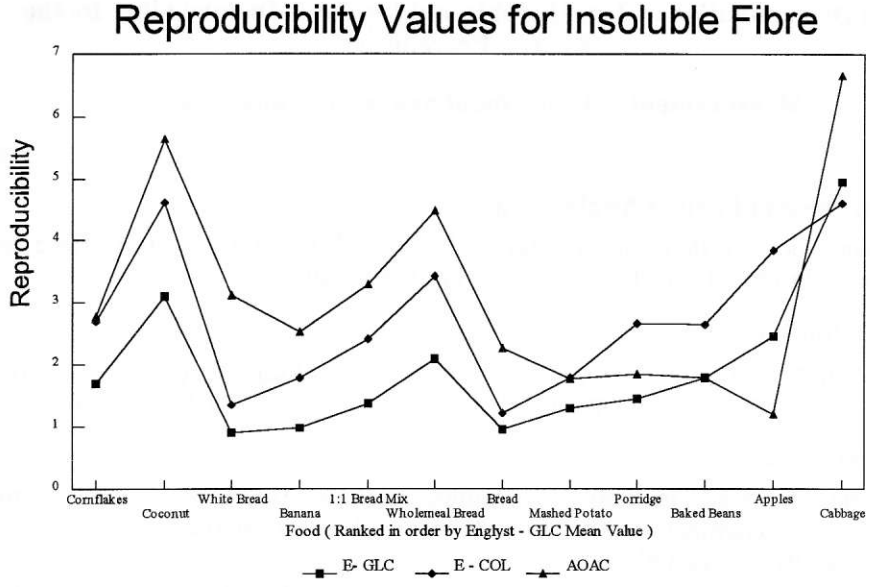


Figure 7:

A comparison of the reproducibility values obtained by the Englyst-GLC, Englyst-colorimetric and Prosky methods for insoluble dietary fibre. Results ordered by mean value obtained using the Englyst-GLC method.

APPENDIX I

Rapid Determination of Total Soluble and Insoluble Dietary Fibre by the Englyst Procedure

Measurement of Constituent Sugars by Colorimetry

1. Scope and Field of Application

The method determines total, soluble and insoluble dietary fibre as non-starch polysaccharides (NSP) in food products.

2. Definition

Dietary fibre, for the purposes of food labelling, is defined as "the polysaccharides of the plant cell wall and chemically related substances".

3. Principle

The Englyst method[13] measures dietary fibre as NSP, using enzymic-chemical methods, and has evolved from the principles laid down by Southgate[15].

In the Englyst procedure, starch is gelatinised and then removed by enzymic digestion. The non-starch polysaccharides are hydrolysed by sulphuric acid, releasing neutral sugars and uronic acids, which are then measured by colorimetry[16]. The method allows the determination of total, soluble and insoluble dietary fibre within an eight-hour working day.

The method is suitable for routine analysis, where details of the separate components of dietary fibre are not required. The technique is simple and rapid, and obviates the need for expensive GLC equipment. Agreement is good between dietary fibre values obtained by GLC and by the colorimetric procedure[16].

4. Contents of the Kit used in the Trial

(adequate for 150 samples)

Instruction leaflet

		Reference samples:	
Amylase (Termamyl)	15 ml	Beans	2g
Amylase (pancreatin)	7.5g	Cabbage	2g
Pullulanase	15 ml	Wheat bran	2g
Sugar mixture	20 ml	White flour	2g
Colour Reagent*	300 ml		

(*See reference 11 for composition)

Keep the reagents and reference samples well-capped. Store the kit at 5 °C. See the table for the dietary fibre content of the reference samples.

Kit developed at:

The Medical Research Council, Dunn Clinical Nutrition Centre

100 Tennis Court Road, Cambridge CB2 1QL, UK.

In collaboration with:

Novo Biolabs, Novo Alle, DK-2880 Bagsvaerd, Copenhagen, Denmark.

5. Reagents

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

5.1 Acetone

5.2 Dimethyl Sulphoxide (DMSO)

5.3 Ethanol, Absolute

5.4 Ethanol, 85 per cent, *V/V*

5.5 Glucose Solution, 1 mg/ml

5.6 Sodium Acetate Buffer, 0.1 mol/l, *p*H 5.2

Dissolve 13.6 g of sodium acetate trihydrate, $CH_3COONa.3H_2O$, and make to a final volume of 1 litre with water. Adjust to *p*H 5.2 with acetic acid, 0.1 mol/l. To stabilise and activate enzymes, add 4 ml of calcium chloride, 1 mol/l, to 1 litre of buffer.

5.7 Sodium Hydroxide, 4.0 mol/l

5.8 Sodium Phosphate Buffer, 0.2 mol/l, *p*H 7.

Adjust Na_2HPO_4, 0.2 mol/l, to *p*H 7 with NaH_2PO_4, 0.2 mol/l.

5.9 Sulphuric Acid, Concentrated

5.10 Sulphuric Acid, 2 mol/l

5.11 Sulphuric Acid, 12 mol/l

Accurately measure 280 ml of water into a 2 litre beaker of good quality. Place the beaker into a bowl of ice-water and slowly add 390 ml of sulphuric acid, concentrated, with stirring.

NB Caution should be taken in making this reagent.

5.12 Sulphuric Acid, 2.4 mol/l

Accurately take 20 ml of sulphuric acid, 12 mol/l, and make to 100 ml with water.

6. Apparatus

6.1 Balance: Accurate to 0.1 mg

6.2 Centrifuge: Capable of exerting 1500 g

6.3 Centrifuge Tubes

Glass centrifuge tubes of 50-60 ml capacity, fitted with Teflon-lined screw tops (24 tubes are suitable for a batch).

6.4 Water-Baths

One capable of maintaining 100 °C; one capable of maintaining temperatures in the range 35 to 50 °C. Each should have a rack that will hold a batch of the glass tubes (**6.3**).

6.5 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.

6.6 Magnetic Stirrers, PTFE-coated: To fit the centrifuge tubes (**6.3**).

6.7 Spectrophotometer

6.8 Vortex Mixer

7. Procedure

7.1 Pre-treatment of Samples

All samples should be finely divided so that representative sub-samples may be taken. Foods with a low water content (<10 per cent) may be milled, and foods with a higher water content may be homogenised wet or milled after freeze-drying.

7.2 Test Samples

Two portions, (a) and (b), of each test sample are required to obtain separate values for total, insoluble and soluble NSP. Portion (a) is used to measure total NSP; portion (b) is used to measure insoluble NSP. Soluble NSP is determined as the difference. The two portions are treated identically throughout the procedure, except for their separate treatment in steps **7.5** and **7.6.**

7.2.1 Sample Weight

Weigh (**6.1**), to the nearest 0.1 mg, between 50 and 1000 mg depending on the water and NSP content of the sample (to give not more than 300 mg of dry matter; e.g. 300 mg is adequate for most dried foods) into 50-60 ml screw-top glass tubes (**6.3**) and add a magnetic stirrer (**6.6**) to each. If the sample is dry (85 to 100 per cent dry matter) and contains less than 5 per cent fat, proceed to step 7.4; otherwise, go to step **7.3.**

7.3 Fat Extraction/Drying of Wet Samples

(Not required for the Kit reference samples)

Add 40 ml of acetone (**5.1**), cap the tubes and mix for 30 minutes using the magnetic stirrer. Centrifuge (**6.2**) at 1000 g to obtain a clear supernatant liquid (5 to 10 minutes) and remove by aspiration as much of the supernatant liquid as possible without disturbing the residue.

Place the tube in a beaker of water at 80 °C on the hot-plate stirrer (**6.5**) and mix the residue until dry. Either use a fume-cupboard or the beaker may be covered and the acetone vapour removed with a water-pump.

7.4 Dispersion and Enzymic Hydrolysis

7.4.1 Treatment with DMSO

Pre-equilibrate sufficient acetate buffer (**5.6**) at 50 °C (8 ml required per sample).

Add 2 ml of DMSO (**5.2**), to the dry sample, cap the tube, and IMMEDIATELY mix the contents using the vortex mixer (**6.8**). It is essential that all the sample is wetted and no material is encapsulated or adhering to the tube wall before proceeding. Vortex mix three or four times during a five-minute period.

Place the tubes (all in one rack) into a boiling water-bath (**6.4**). Remove the rack of tubes after 30 seconds and vortex mix the contents of each tube. Replace the rack of tubes into the boiling water-bath and leave it there for 30 minutes. During this period, prepare the following enzyme solutions (the volumes given are suitable for 24 samples).

7.4.1.1 Enzyme Solution I

Take 2.5 ml of Termamyl (Kit), make to 200 ml with the pre-equilibrated acetate buffer, mix, and keep it in the 50 °C water-bath.

7.4.1.2 Enzyme Solution II

Take 1.2 g of pancreatin (Kit) into a 50 ml tube, add 12 ml of water, vortex mix initially and then mix for 10 minutes with a magnetic stirrer. Vortex mix again, then centrifuge for 10 minutes. Take 10 ml of the (cloudy) supernatant, add 2.5 ml of pullulanase (Kit) and vortex mix. Keep the solution at room temperature.

7.4.2 Treatment with Enzymes

Remove ONE TUBE AT A TIME, vortex mix, uncap and IMMEDIATELY add 8 ml of enzyme solution I, cap the tube, vortex thoroughly, ensuring that no material adheres to the tube wall, and replace in the boiling water-bath. Leave the tubes there for 10 minutes, timed from the last addition of enzyme.

Transfer the rack of tubes to the 50 °C water-bath. After 3 minutes, add 0.5 ml of enzyme solution II to each tube and mix the contents thoroughly to aid distribution of the enzyme throughout the sample. Replace the tubes in the 50 °C water-bath and leave them there for 30 minutes. Transfer the rack of tubes to the boiling water-bath and leave them there for 10 minutes.

(See also Appendix III)

7.5 Precipitation and Washing of the Residue for Measurement of Total Dietary Fibre

TEST SAMPLE PORTION (a) ONLY

7.5.1 Cool the sample by placing in water at room temperature. Add 40 ml of ethanol, absolute (**5.3**), mix well by repeated inversion, then leave in ice-water for 30 minutes. Centrifuge (**6.2**) at 1500 g to obtain a clear supernatant liquid (5 to 10 minutes). Remove by aspiration as

much of the supernatant liquid as possible, without disturbing the residue, and discard it.

7.5.2 Add approximately 10 ml of ethanol, 85 per cent (**5.4**), and vortex mix. Make to 50 ml with ethanol, 85 per cent, mix by inversion, then use the magnetic stirrer to form a suspension of the residue. Centrifuge and remove the supernatant liquid as above. Repeat this stage using 50 ml of ethanol, absolute.

7.5.3 Add 20 ml of acetone to the residue and vortex mix, then use the magnetic stirrer to form a suspension. Centrifuge and remove the supernatant liquid as above.

7.5.4 Place the tube in a beaker of water at 80 °C on the hot-plate stirrer and mix the residue until dry. (It is essential that the residue and tube are completely free of acetone.) Either use a fume-cupboard or the beaker may be covered and the acetone vapour removed with a water-pump. (If aggregation occurs during drying, disperse the sample using the vortex mixer. This is best done before the sample is completely dry.)

7.6 Extraction and Washing of the Residue for Measurement of Insoluble Dietary Fibre

TEST SAMPLE PORTION (b) ONLY

7.6.1 After the treatment with enzymes in step 7.4, add 40 ml of phosphate buffer (**5.8**). Place the capped tubes in the boiling water-bath for 30 minutes. Mix continuously or three times during this period. Remove the tubes to water at room temperature and leave for 10 minutes. Centrifuge and remove the supernatant liquid as described in step **7.5.1.**

7.6.2 Add approximately 10 ml of water and vortex mix. Make to 50 ml with water, mix by inversion, then use the magnetic stirrer to form a suspension of the residue. Centrifuge and remove the supernatant liquid as above. Repeat this stage using 50 ml of ethanol, absolute.

7.6.3 Proceed as described for step **7.5.3** and step **7.5.4**.

7.7 Acid Hydrolysis of the Residue from Enzymic Digestion

Add 5 ml of sulphuric acid, 12 mol/l (**5.11**) to the dry residue and immediately vortex mix. It is essential to ensure that all the material is wetted. Leave the tubes at 35 °C for 1 hour with occasional or continuous mixing to disperse the cellulose.

Add 25 ml of water rapidly and vortex mix. Place into a boiling water-bath (**6.4**) and leave for 1 hour, timed from when boiling recommences; stir continuously or once after 10 minutes. Cool the tubes in tap water.

(See also Appendix II)

7.8 Preparation of Standard Sugar Solutions

Take 1000 µl of the Kit sugar mixture into a glass tube, add 5000 µl of sulphuric acid, 2.4 mol/l (**5.12**), and mix to give 6 ml of 4 mg sugars/ml standard solution in sulphuric acid, 2 mol/l. (The value of 4

mg sugars/ml is based on a content of 14 mg of arabinose, 7 mg of glucose and 3 mg of galacturonic acid per 6 ml of the Kit sugar mixture as diluted here. The actual amount of each sugar is 2 per cent less, to account for losses during the hydrolysis in step **7.7**.)

Further standards of 2 and of 1 mg sugars/ml may be prepared by double dilution of the 4 mg sugars/ml standard and used for the construction of a standard curve to test for linearity of response.

For routine analysis, only the 4.0 mg/ml standard is required.

7.9 Measurement of Total Reducing Sugars

Place into separate tubes 1 ml of blank solution (sulphuric acid, 2 mol/l (5.10)), 1 ml of each of the standard solutions from step **7.8**, and 1 ml of the hydrolysate from step **7.7.** Samples with more than about 40 g NSP per 100 g dry matter should be diluted with sulphuric acid, 2 mol/l.

Add 0.1 ml of glucose solution, 1 mg/ml (**5.5**), and 1 ml of sodium hydroxide, 4.0 mol/l (**5.7**), to each tube and vortex mix.

Add 2 ml of the Kit colour reagent solution to each tube and vortex mix. Place the tubes, ALL AT THE SAME TIME, into a briskly boiling water-bath (**6.4**) for 15 minutes. Cool in water to room temperature. Add 25 ml of water and mix well by inversion.

Measure the absorbance in the spectrophotometer (**6.7**) at 530 nm. A straight line should be obtained if the absorbance values for the standards are plotted against concentration. The colorimetic reaction is suitable for automation[11]. (See also Appendix I)

8. Calculation of Total, Soluble and Insoluble Dietary Fibre

The amount of total DF (portion (a) and of insoluble DF (portion (b)) in g/100 g of sample, is calculated as:

$$\frac{A_T \times V_T \times D \times F \times C \times 100}{A_S \times W_T} \times 0.89$$

where :

A_T is the absorbance of the test solution;

V_T is the total volume of the test solution (here, 30 ml);

D is the dilution of the test solution;
$D = 1$ if no dilution in step 7.9;

A_S is the absorbance of the 4 mg/ml standard;

C is the concentration (in mg sugars/ml) of the standard used (here, $C = 4$);

W_T is the weight (in mg) of sample taken for analysis;

F is the factor correcting the difference between the composition of monosaccharides in the Kit sugar mixture and that in NSP of various types of plant foods.

For the calculation of total DF: cereals, $F = 0.97$; fruit and non-starchy vegetables, $F = 1.03$; starchy vegetables and unknown samples, $F = 1$.

For the calculation of insoluble DF: $F = 1$ except for cereals, where $F = 0.97$; 0.89 is the factor for converting the experimentally determined monosaccharides to polysaccharides.

The amount of soluble dietary fibre is calculated as the difference between total and insoluble dietary fibre.

9. Breaks in the Procedure

The whole analysis may be completed in one day if desired. However, the procedure may be halted at either of the following stages.

9.1 After precipitation, washing and drying the starch-free residue in steps **7.5** and **7.6**. The residue may be stored for long periods.

9.2 After the hydrolysis with sulphuric acid, 2 mol/l, in step **7.7**. The hydrolysate may be kept at 5 °C for 24 hours.

10. Trouble Shooting

Sample Type	Likely cause	Cure/Prevention
1. Large Variation between replicate analysis	(a) Inaccurate pipetting of the kit sugar mixture in step **7.8** (b) Incomplete removal of the acetone in step **7.5.4**	(a) Test/calibrate dispensers by weighing ml replicates of water (b) Ensure an acetone free powder in step **7.5.**
2. Values too high and variable for replicates	Incomplete wetting of sample with DMSO in step **7.4.1**	Mix vigorously immediately after addition of DMSO
3. Value too low and variable for replicates	Incomplete wetting of sample with sulphuric acid in step **7.7**	Vortex mix vigorously before and immediately after the addition of sulphuric acid, and at intervals during the incubation.
4. No colour produced for standards and/or test samples	Error in preparation of sulphuric acid or sodium hydroxide solutions	Make new reagents Test that the solution is neutral/alkaline before adding colour reagent solution.

Dietary Fibre in the Four Reference Samples

	Results using main procedure (g/100g sample)	Results obtained by separate measurement of uronic acids (g/100g sample)		
		Total	Neutral Sugars	Uronic Acids
Haricot Beans				
Soluble DF	7.7	8.1	6.2	1.9
Insoluble DF	11.2	11.4	9.9	1.5
Total DF	18.9	19.5	16.1	3.4
Spring Cabbage				
Soluble DF	11.2	11.9	5.8	6.1
Insoluble DF	11.9	11.6	11.1	0.5
Total DF	23.1	23.5	16.9	6.6
Wheat Bran				
Soluble DF	4.7	4.7	4.6	0.1
Insoluble DF	32.1	32.3	31.1	1.2
Total DF	36.8	37.0	35.7	1.3
White Flour				
Soluble DF	1.4	1.4	1.4	t
Insoluble DF	2.4	2.4	2.3	0.1
Total DF	3.8	3.8	3.7	0.1

Englyst Procedure
For Rapid Determination of Total, Soluble and Insoluble Dietary Fibre by Colorimetry

SAMPLE

↓

Add 2 ml DMSO

↓

30 min at 100°C

↓

Add 8 ml enzyme solution I

↓

10 min at 100°C

↓

Add 0.5 ml enzyme solution II

↓

30 min at 50°C/10 min at 100°C

↓

Add 40 ml ethanol

↓

0.5 h at 0°C

↓

Centrifuge
Wash with ethanol, 85 and 100 per cent
Dry with acetone

↓

Add 5 ml H_2SO_4, 12 mol/l

↓

1 h at 35°C

↓

Add 25 ml water

↓

1 h at 100°C

↓

Add to 1 ml:
0.1 ml glucose solution
1 ml NaOH, 4 mol/l
2 ml Kit colour reagent

↓

15 min at 100°C

↓

Add 25 ml water

↓

Read the absorbance at 530nm
Calculate total dietary fibre

Soluble DF = Total DF - Insoluble DF.

For measurement of insoluble dietary fibre, replace the 40 ml of ethanol with 40 ml of *p*H 7 buffer and extract for 0.5h at 100°C.

APPENDIX II

Determination of Total, Soluble and Insoluble Dietary Fibre by the Englyst Procedure. Measurement of Constituent Sugars by Gas-Liquid Chromatography

1. Scope and Field of Application

The method determines total, soluble and insoluble dietary fibre as non-starch polysaccharides (NSP) in food products.

2. Definition

Dietary fibre, for the purposes of food labelling, is defined as "the polysaccharides of the plant cell wall and chemically related substances".

3. Principle

The Englyst method[13] measures dietary fibre as NSP, using enzymic-chemical methods, and has evolved from the principles laid down by Southgate[15].

In the Englyst procedure, starch is gelatinised and then removed by enzymic digestion. The non-starch polysaccharides are hydrolysed by sulphuric acid, releasing neutral sugars, which are measured by chromatography (GLC), and uronic acids, which are measured by colorimetry. The GLC technique used in this method allows the determination of total, soluble and insoluble dietary fibre, and these fractions are further characterised by measurement of the component sugars. Using this technique, the measurement of dietary fibre and its components can be carried out in one and a half working days.

4. Contents of the Kit used in the Trial

(adequate for 150 samples)

Instruction leaflet		Reference samples:	
Amylase (Termamyl)	15 ml	Beans	2g
Amylase (pancreatin)	7.5g	Cabbage	2g
Pullulanase	15 ml	Wheat bran	2g
Sugar mixture	20 ml	White flour	2g

Keep the reagents and reference samples well-capped. Store the kit at 5°C. See the table for the dietary fibre content of the reference samples.

Kit developed at:

The Medical Research Council, Dunn Clinical Nutrition Centre

100 Tennis Court Road, Cambridge CB2 1QL, UK.

In collaboration with:

Novo Biolabs, Novo Alle, DK-2880 Bagsvaerd, Copenhagen, Denmark.

5. Reagents

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

5.1 Acetic Acid, Glacial

5.2 Acetic Anhydride

5.3 Acetone

5.4 Ammonium Hydroxide, 12.5 mol/l

5.5 Ammonium Hydroxide/Sodium Borohydride Solution

A solution of ammonium hydroxide, 2mol/l, containing 200 mg of sodium borohydride, $NaBH_4$, per ml. Prepare immediately before use.

5.6 Benzoic Acid Solution, 50 per cent saturated

Dilute saturated benzoic acid solution 1: 1, *V/V*, with water

5.7 Bromophenol Blue Solution, 0.04 per cent *w/V*

5.8 Dimethylphenol Solution

Dissolve 0.1 g of 3,5-dimethylphenol, $(CH_3)_2C_6H_3OH$, in 100 ml of glacial acetic acid (**5.1**)

5.9 Dimethyl Sulphoxide (DMSO)

5.10 Ethanol, Absolute

5.11 Ethanol, 85 per cent *V/V*

5.12 GLC Internal Standard Solution, 1 mg/ml

Weigh 500 mg of allose (dried to constant weight under reduced pressure with phosphorus pentoxide) to the nearest 0.1 mg. Make to 500 ml with 50 per cent saturated benzoic acid (**5.6**) to give a 1 mg/ml solution. Stable for several months at room temperature.

5.13 1-Methylimidazole

5.14 Octan-2-*ol*

5.15 Potassium Hydroxide, 7.5 mol/l

5.16 Sodium Acetate Buffer, 0.1 mol/l, *p*H 5.2

Dissolve 13.6 g of sodium acetate trihydrate, $CH_3COONa.3H_2O$, and make to 1 litre with water. Adjust to *p*H 5.2 with acetic acid, 0.1 mol/l. To stabilise and activate enzymes, add 4 ml of calcium chloride, 1 mol/l, to 1 litre of buffer.

5.17 Sodium Chloride/Boric Acid Solution

Dissolve 2 g of sodium chloride, NaC1, and 3 g of boric acid, H_3BO_3, in 100 ml of water

5.18 Sodium Phosphate Buffer, 0.2 mol/l, *p*H 7

Adjust Na_2HPO_4, 0.2 mol/l, to *p*H 7 with NaH_2PO_4, 0.2 mol/l

5.19 Sulphuric Acid, Concentrated

5.20 Sulphuric Acid, 2 mol/l

5.21 Sulphuric Acid, 12 mol/l

Accurately measure 280 ml of water into a 2 litre beaker of good quality. Place the beaker in a bowl of ice-water and slowly add 390 ml of concentrated sulphuric acid with stirring.

NB Caution should be taken in making this reagent

5.22 Sulphuric Acid, 2.4 mol/l

Accurately take 20 ml of sulphuric acid, 12 mol/l, and make to 100 ml with water

6. Apparatus

6.1 Balance: Accurate to 0.1 mg

6.2 Centrifuge: Capable of exerting 1500 g

6.3 Centrifuge Tubes

Glass centrifuge tubes of 50-60 ml capacity, fitted with Teflon-lined screw tops (24 tubes are suitable for a batch).

6.4 GLC Chromatograph

GLC chromatograph fitted with flame ionisation detector and, preferably, auto-injector and computing integrator

6.5 GLC Column

Supelco SP-2330 wide-bore capillary column (30m × 0.75 mm: Supelco lot no 2-3751)

6.6 Heating Block

6.7 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.

6.8 Magnetic Stirrers, PTFE-coated: To fit the centrifuge tubes (**6.3**).

6.9 Spectrophotometer

6.10 Vortex Mixer

6.11 Water-baths

One capable of maintaining 100°C; one capable of maintaining temperatures in the range 35°C to 50°C. Each should have a rack that will hold a batch of the glass tubes (**6.3**)

7. Procedure

7.1 Pre-treatment of Samples

All samples should be finely divided so that representative sub-samples (dry or wet) may be taken. Foods with a low water content (<10 per cent) may be milled, and foods with a higher water content may be homogenised wet or milled after freeze-drying.

7.2 Test Samples

Two portions, (a) and (b), of each test sample are required to obtain separate values for total, insoluble and soluble NSP. Portion (a) is

used to measure total NSP; portion (b) is used to measure insoluble NSP. Soluble NSP is determined as the difference. The two portions are treated identically throughout the procedure, except for their separate treatment in steps **7.5** and **7.6**.

7.2.1 Sample Weight

Weigh (**6.1**), to the nearest 0.1 mg, between 50 and 1000 mg depending on the water and NSP content of the sample (to give not more than 300 mg of dry matter; e.g. 300 mg is adequate for most dried foods) into 50-60 ml screw-top glass tubes (**6.3**) and add a magnetic stirrer (**6.8**) to each. If the sample is dry (85 to 100 per cent dry matter) and contains less than 5 per cent fat, proceed to step **7.4**; otherwise, go to step **7.3**.

7.3 Fat Extraction/Drying of Wet Samples

(Not required for the Kit reference samples)

Add 40 ml of acetone (**5.3**), cap the tubes and mix for 30 minutes using the magnetic stirrer. Centrifuge at 1000 g to obtain a clear supernatant liquid (5 to 10 minutes) and remove by aspiration as much of the supernatant liquid as possible without disturbing the residue.

Place the tube in a beaker of water at 80 °C on the hot-plate stirrer (**6.7**) and mix the residue until dry. Either use a fume-cupboard or the beaker may be covered and the acetone vapour removed with a water-pump.

7.4 Dispersion and Enzymic Hydrolysis

7.4.1 Treatment with DMSO

Pre-equilibrate sufficient acetate buffer (**5.16**) at 50 °C (8 ml required per sample).

Add 2 ml of DMSO (**5.9**) to the dry sample, cap the tube, and IMMEDIATELY mix the contents using the vortex mixer (**6.10**). It is essential that all the sample is wetted and no material is encapsulated or adhering to the tube wall before proceeding. Vortex mix three or four times during a five-minute period.

Place the tubes (all in one rack) into a boiling water-bath (**6.11**). Remove the rack of tubes after 30 seconds and vortex mix the contents of each tube. Replace the rack of tubes into the boiling water-bath and leave it there for 30 minutes. During this period, prepare the following enzyme solutions (the volumes given are suitable for 24 samples).

7.4.1.1 Enzyme Solution I

Take 2.5 ml of Termamyl (Kit), make to 200 ml with the pre-equilibrated acetate buffer, mix, and keep it in the 50 °C water-bath.

7.4.1.2 Enzyme Solution II

Take 1.2 g of pancreatin (Kit) into a 50 ml tube, add 12 ml of water, vortex mix initially and then mix for 10 minutes with a magnetic stirrer. Vortex mix again, then centrifuge for 10 minutes. Take 10 ml

of the (cloudy) supernatant, add 2.5 ml of pullulanase (Kit) and vortex mix. Keep the solution at room temperature.

7.4.2 Treatment with Enzymes

Remove ONE TUBE AT A TIME, vortex mix, uncap and IMMEDIATELY add 8 ml of enzyme solution I, cap the tube, vortex thoroughly, ensuring that no material adheres to the tube wall, and replace in the boiling water-bath. Leave the tubes there for 10 minutes, timed from the last addition of enzyme.

Transfer the rack of tubes to the 50 °C water-bath. After 3 minutes, add 0.5 ml of enzyme solution II to each tube and mix the contents thoroughly to aid distribution of the enzyme throughout the sample. Replace the tubes in the 50 °C water-bath and leave them there for 30 minutes. Mix the contents of each tube continuously or after 10 minutes, 20 minutes and 30 minutes. Transfer the rack of tubes to the boiling water-bath and leave them there for 10 minutes.

7.5 Precipitation and Washing of the Residue for Measurement of Total Dietary Fibre

TEST SAMPLE PORTION (a) ONLY

7.5.1 Cool the sample by placing in water at room temperature. Add 40 ml of ethanol, absolute (**5.10**), mix well by repeated inversion, then leave in ice-water for 30 minutes. Centrifuge (**6.2**) at 1500 g to obtain a clear supernatant liquid (5 to 10 minutes). Remove by aspiration as much of the supernatant liquid as possible, without disturbing the residue, and discard it.

7.5.2 Add approximately 10 ml of ethanol, 85 per cent (**5.11**), and vortex mix. Make to 50 ml with ethanol, 85 per cent, mix by inversion, then use the magnetic stirrer to form a suspension of the residue. Centrifuge and remove the supernatant liquid as above. Repeat this stage using 50 ml of ethanol, absolute.

7.5.3 Add 20 ml of acetone to the residue and vortex mix, then use the magnetic stirrer to form a suspension. Centrifuge and remove the supernatant liquid as above.

7.5.4 Place the tube in a beaker of water at 80 °C on the hot-plate stirrer and mix the residue until dry. (It is essential that the residue and tube are completely free of acetone.) Either use a fume-cupboard or the beaker may be covered and the acetone vapour removed with a water-pump. (If aggregation occurs during drying, disperse the sample using the vortex mixer. This is best done before the sample is completely dry.)

7.6 Extraction and Washing of the Residue for Measurement of Insoluble Dietary Fibre

TEST SAMPLE PORTION (b) ONLY

7.6.1 After the treatment with enzymes in step **7.4**, add 40 ml of phosphate buffer (**5.18**). Place the capped tubes in the boiling water-bath for 30 minutes. Mix continuously or three times during this period. Remove the tubes to water at room temperature and leave

for 10 minutes. Centrifuge and remove the supernatant liquid as described in step **7.5.1**.

7.6.2 Add approximately 10 ml of water and vortex mix. Make to 50 ml with water, mix by inversion, then use the magnetic stirrer to form a suspension of the residue. Centrifuge and remove the supernatant liquid as above. Repeat this stage using 50 ml of ethanol, absolute.

7.6.3 Proceed as described for step **7.5.3** and step **7.5.4.**

7.7 Acid Hydrolysis of the Residue from Enzymic Digestion

Add 5 ml of sulphuric acid, 12 mol/l (**5.21**) to the dry residue and immediately vortex mix. It is essential to ensure that all the material is wetted. Leave the tubes at 35 °C for 1 hour with occasional or continuous mixing to disperse the cellulose.

Add 25 ml of water rapidly and vortex mix. Place into a boiling water-bath and leave for 1 hour, timed from when boiling recommences; stir continuously or once after 10 minutes. Cool the tubes in tap water.

7.8 Standard Sugar Mixture for Calibration

Place 2000 µl of the Kit sugar mixture into a 50-60 ml glass tube, add 10 ml of sulphuric acid, 2.4 mol/l (**5.22**) and mix. Treat 3 ml of this mixture in parallel with the test samples in step 7.9.

7.9 Preparation of Alditol Acetate Derivatives

Prepare sufficient (0.2 ml is required for each tube) of the ammonium hydroxide/sodium borohydride solution (**5.5**)

Add 1000 µl of internal standard (1 mg allose/ml, (**5.12**)) to 3000 µl of the cooled hydrolysates from step **7.7** and to 3000 µl of the standard sugar mixture from step **7.8**; vortex mix.

Place the tubes in ice-water, add 1 ml of ammonium hydroxide, 12.5 mol/l (**5.4**), and vortex mix. Test that the solution is alkaline (add a little more ammonium hydroxide if necessary), then add approximately 5 µl of the antifoam agent octan-2-*ol* (**5.14**) and 0.2 ml of the ammonium hydroxide/sodium borohydride solution, vortex mix.

Leave the tubes in a heating block (**6.6**) or water-bath at 40°C for 30 minutes then remove and add 0.4 ml of glacial acetic acid (**5.1**), mix again. Remove 0.5 ml to a 30 ml glass tube; add 0.5 ml of 1-methylimidazole (**5.13**) and 5 ml of acetic anhydride (**5.2**). Vortex mix then leave for 10 minutes.

Add 0.9 ml of ethanol, absolute, vortex mix and leave for 5 minutes. Add 10 ml of water, vortex mix and leave for 5 minutes. Add 0.5 ml of bromophenol blue solution (**5.7**). Place the tubes in ice-water and add 5 ml of potassium hydroxide, 7.5 mol/l (**5.15**), a few minutes later add a further 5 ml of potassium hydroxide, 7.5 mol/l, cap the tubes and mix by inversion.

Leave until the separation into two phases is complete (10 to 15 minutes) or centrifuge for a few minutes. Draw the upper phase into the tip of an automatic pipette; if any of the blue phase is included,

allow it to separate then run it out of the tip before transferring the upper phase alone to a small (auto-injector) vial.

7.10 GLC measurement of Neutral Sugars

Carry out conventional GLC (**6.4**) measurement of the neutral sugars. Inject 0.5 to 1 µl of the alditol acetate derivatives prepared in step **7.9**

GLC conditions:

Injector temperature	275°C
Column temperature	220°C
Detector temperature	275°C
Carrier gas	Helium, 8 ml/min

The actual amount of each sugar other than allose is less, to correct for the percentage recovery after the treatment with acid in step **7.7** (rhamnose, 93 per cent; fucose, 99 per cent; arabinose, 95 per cent; xylose, 89 per cent; mannose, 92 per cent; galactose, 94 per cent; glucose, 94 per cent; galacturonic acid, 87 per cent).

8. Calculation of Neutral Sugars

The amount of individual sugars (in g/100 g of sample) is calculated as

$$\frac{A_T \times W_1 \times 100 \times R_F}{A_I \times W_T} \times 0.89$$

where

A_T and A_I are the peak areas of the test sample and the internal standard, respectively;

W_T is the weight (in mg) of the test sample;

W_I is the weight (in mg; here 10 mg) of the internal standard;

R_F is the response factor for individual sugars obtained from the calibration run with the sugar mixture treated in parallel with the test samples;

and 0.89 is a scale factor for converting the experimentally determined values for monosaccharides to polysaccharides.

There is incomplete hydrolysis of any rhamnose. This is corrected for by applying a factor of 1.5 to the rhamnose values as determined experimentally. In practice, only traces of rhamnose are present in food products.

9. Measurement of Uronic Acids

9.1 Standard Solutions

The standard sugar mixture in sulphuric acid, 2 mol/l, prepared in step **7.8** (12 ml) contains, for the purposes of calculation, 500 µg of galacturonic acid/ml.

To prepare the standard solutions, take 0.5 ml, 1.0 ml, 2.0 ml and 3.0 ml of the sugar mixture into separate tubes and make to 10.0 ml with sulphuric acid, 2 mol/l (5.20), to give standards of 25, 50, 100 and 150 µg of galacturonic acid/ml.

Only the 100 µg/ml standard is required for routine analysis (it may be kept at 5 °C for several weeks).

9.2 Measurement

Place into separate tubes (40 - 50 ml capacity) 0.3 ml of blank solution (sulphuric acid, 2 mol/l), 0.3 ml of each of the standard solutions and 0.3 ml of the test sample hydrolysates, diluted if necessary (with sulphuric acid, 2 mol/l) to contain no more than 150 µl of uronic acids per ml (e.g. no dilution for flour, 1:2 for bran, 1:5 for most fruits and vegetables). Add 0.3 ml of sodium chloride/boric acid solution (**5.17**) and mix.

Add 5 ml of sulphuric acid, concentrated (**5.19**) and vortex mix. Place the tubes in a heating block at 70 °C and leave for 40 minutes. Remove the tubes and cool to room temperature in water (the tubes may be kept in the water for up to 1 hour).

Add 0.2 ml of dimethylphenol solution (**5.8**) and vortex mix immediately. Between 10 and 15 minutes later, measure the absorbance at 400 nm and at 450 nm in the spectrophotometer (**6.9**) against the blank solution. Subtract the reading at 400 nm from that at 450 nm, to correct for the interference from hexoses.

A straight line should be obtained if the differences for the standards are plotted against concentration.

10. Calculation of Uronic Acids

10.1 The amount of uronic acids (in g/100 g of sample) is calculated as:

$$\frac{A_T \times V_T \times D \times 10}{A_S \times W_T} \times 0.89$$

where

A_T is the difference in absorbance of the test solution;

V_T is the total volume of test solution (here 30 ml);

D is the dilution of the test solution;

A_S is the difference in absorbance of the 100 µg/ml standard;

W_T is the weight (in mg) of the test sample;

0.89 is a scale factor for converting the experimentally determined values for monosaccharides to polysaccharides.

10.2 Calculation of Total, Soluble and Insoluble Dietary Fibre (DF)

The amount of total, soluble and insoluble DF, in g/100 g of sample, is calculated as:

Total DF = Neutral sugars calculated for portion (a) [step 8]
+ Uronic acids calculated for portion (a) [step 10]
Insoluble DF = Neutral sugars calculated for portion (b) [step 8]
+ Uronic acids calculated for portion (b) [step 10]
Soluble DF = Total DF - Insoluble DF

11. Breaks in the Procedure

The procedure may be halted at any of the following stages.

After precipitation, washing and drying the starch-free residue in steps **7.5** and **7.6**. The residue may be stored for long periods.

After the hydrolysis with sulphuric acid, 2 mol/l, in step **7.7.** The hydrolysate may be kept at 5 °C for 24 hours.

After acidification of the reduced samples in step **7.9**. The samples may be stored at room temperature for two or three days.

After acetylation and transfer to small vials in step **7.9**. The samples may be kept at room temperature for two to three days before analysis by GLC.

The acid hydrolysate in step **7.9** may be kept at 5 °C for several weeks before the measurement of uronic acids.

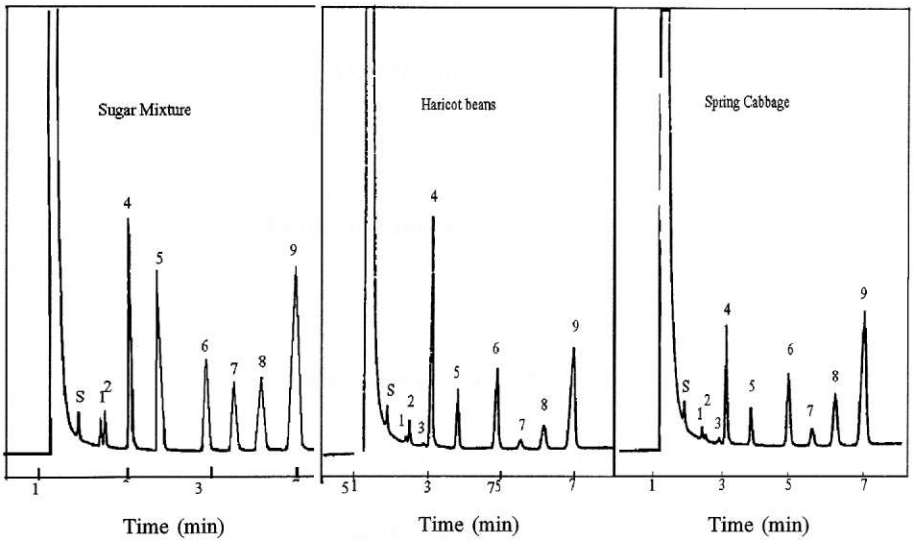
12. Trouble Shooting

Symptom	Likely cause	Cure/Prevention
1. Extra peaks on the chromatogram	Incomplete reduction of monosaccharides in step **7.9**	Ensure alkaline *p*H before adding sodium borohydride. Replace old sodium borohydride. Do not compensate for loss of activity by adding more sodium borohydride
2. Large variation between replicate analysis	Inaccurate pipetting of the internal standard	Test/calibrate dispensers by weighing 2 ml replicates of water
3. Response Factors not reproducible	Inaccurate pipetting of the Kit sugar mixture in step **7.8**	Test/calibrate dispensers by weighing 2 ml replicates of water
4. Values for glucose too high and variable for replicates	Incomplete wetting of sample with DMSO in step **7.4.1**	Vortex mix vigorously immediately after addition of DMSO and at intervals during the incubation

Dietary Fibre in the Four Reference Samples

	Total (g/100 g)	Composition (g/100g)							
	Sample	Rha	Fuc	Ara	Xyl	Man	Gal	Glu	U.Ac
Haricot Beans									
Soluble DF	7.6	0.2	0.3	3.0	0.6	0.1	0.7	0.8	1.9
Insoluble DF	11.0	0.1	0.1	3.2	1.2	0.3	0.4	4.2	1.5
Total DF	18.6	0.3	0.4	6.2	1.8	0.4	1.1	5.0	3.4
Spring Cabbage									
Soluble DF	11.1	0.5	t	2.4	0.2	0.1	1.5	0.3	6.1
Insoluble DF	11.8	0.1	0.1	0.6	1.0	0.6	0.9	8.0	0.5
Total DF	22.9	0.6	0.1	3.0	1.2	0.7	2.4	8.3	6.6
Wheat Bran									
Soluble DF	4.3	t	t	1.0	2.3	t	0.3	0.6	0.1
Insoluble DF	31.4	t	t	7.1	13.1	0.3	0.6	9.1	1.2
Total DF	35.7	t	t	8.1	15.4	0.3	0.9	9.7	1.3
White Flour									
Soluble DF	1.4	t	t	0.4	0.5	t	0.2	0.3	t
Insoluble DF	2.1	t	t	0.6	0.9	0.1	0.1	0.3	0.1
Total DF	3.5	t	t	1.0	1.4	0.1	0.3	0.6	0.1

GLC Chromatograms of the Kit Sugar Mixture
and Two Reference Samples



SP-2330 Wide-bore capillary column (30 m × 0.75 mm).
Column temperature 220°C.
Carrier gas, helium (8 ml/min).
The peaks are labelled:

S, solvent; 1, rhamnose; 2, fucose; 3, ribose (not part of DF);
4, xylose; 5, arabinose; 6, allose (internal standard);
7, mannose; 8, galactose; 9, glucose.

Determination of Total, Soluble and Insoluble Dietary Fibre by the Englyst GLC Procedure: Flow Diagram

SAMPLE

|

Add 2 ml DMSO

|

30 min at 100°C

|

Add 8 ml enzyme solution I

|

10 min at 100°C

|

Add 0.5 ml enzyme solution II

|

30 min at 50°C, 10 min at 100°C

|

Add 40 ml ethanol

|

0.5 h at 0°C

|

Centrifuge
Wash with ethanol, 85 and 100 per cent
Dry with acetone

|

Add 5 ml H_2SO_4, 12 mol/l

|

1 h at 35°C

|

Add 25 ml water

|

1 h at 100°C

|

HYDROLYSATE

Flow Diagram - Continued

HYDROLYSATE

NEUTRAL SUGARS	**URONIC ACIDS**
Add to 3 ml; 1 ml internal standard, 1 ml NH_4OH, 12.5 mol/l; 5µl octan-2-*ol*, 0.2 ml $NaBH_4$ solution	**Add (to 0.3ml);0.3 ml $NaCl/H_3BO_4$, 5 ml H_2SO_4**
0.5 h at 40°C	40 min at 70°C
Add 0.4 ml acetic acid	**Add 0.2 ml dimethylphenol**
Add (to 0.5 ml); 0.5 ml 1-methylimidazole, 5 ml acetic anhydride	
Leave 10 min	Leave 10 min
Add 0.9 ml ethanol	
Leave 5 min	
Add 10 ml water, 2 × 5 ml KOH, 7.5 mol/l	
Use top phase for GLC measurement of neutral sugars	**Read absorbance at 400nm and 450nm** **Calculate uronic acids**

Total DF = Neutral sugars + Uronic acids.

Soluble DF = Total DF - Insoluble DF.

For measurement of insoluble DF, replace the 40 ml ethanol with 40 ml *p*H 7 buffer and extract for 0.5h at 100°C.

Appendix III
Prosky Procedure for the Determination of Insoluble and Soluble Dietary Fibre in Foods (Enzymatic - Gravimetric Method)

1. Principle

Duplicate samples of dried foods, fat-extracted if containing> 10% fats, are gelatinised with Termamyl (heat-stable-alpha-amylase), and then enzymatically digested with protease and amyloglucosidase to remove protein and starch.

1.1. Insoluble Dietary Fibre

The residue is filtered and washed with distilled H_2O. The filtrate and wash are saved. The residue (insoluble dietary fibre) is washed with 95% EtOH and acetone. After drying, the residue is weighed. One of the duplicates is analysed for protein, and the other is incinerated at 525 °C and the ash determined.

Insoluble dietary fibre is the weight of the residue less the weight of the protein and ash present.

1.2 Soluble Dietary Fibre

Four volumes of 95% EtOH are added to the combined filtrate and water washing to precipitate soluble dietary fibre. The precipitate is filtered and washed with 78% ethanol, 95% ethanol and acetone. After drying, the residue is weighed. One of the duplicates is analysed for protein, and the other is incinerated at 525 °C and the ash determined.

Soluble dietary fibre is the weight of the residue less the weight of the protein and ash present.

2. Apparatus

2.1 Fritted crucible - Porosity No 2 (Pyredx No 32940, coarse ASTM 40-60 µm; or Corning No 36060 buchner, fritted disk, Pyrex, 60 mL, ASTM 40-60 µm). Clean thoroughly, heat 1 h at 525 °C, and soak and then rinse in H_2O. Add ca 0.5 g Celite to air-dried crucibles and dry at 130 °C to consistant weight (< 1 h). Cool and store in desiccator until used.

2.2 Vacuum source - Vacuum pump or aspirator equipped with inline double vac flask to prevent contamination in case of H_2O backup.

2.3 Vacuum oven - 70 °C. Alternatively, 105 °C air oven can be used.

2.4 Desiccator

2.5 Muffle furnace

2.6 Water baths - (1) Boiling. (2) Constant temperature. - Adjustable to 60 °C, with either multistation shaker or multistation magnetic stirrer to provide consistent agitation of digestion flasks during enzymatic hydrolysis.

2.7 Beakers - Tall-form, 400 ml.
- Tall-form, 600 ml (or Erlenmeyers).

2.8 Balance - Analytical, capable of weighing to 0.1 mg.

2.9 *p*H meter - Standardised with *p*H 7 and *p*H 4 buffers.

2.10 Balance - Analytical, capable of weighing to 0.1 mg.

3. Reagents

3.1 95% Ethanol - *V/V*, technical grade.

3.2 78% Ethanol - Mix one volume of distilled H_2O with four volumes of 95% EtOH.

3.3 Acetone - Reagent grade.

3.4 Phosphate buffer - 0.08M, *p*H 6.0. Dissolve 1.400 g Na phosphate dibasic, anhydrous. (Na_2HPO_4) (or 1.753 g dihydrate) and 9.68 g Na phosphate monobasic ($NaH_2PO_4.H_20$) (or 10.04 g dihydrate) in ca 700 ml H_2O. Dilute to 1 l with H_2O. Check *p*H with pH meter.

3.5 Termamyl (heat-stable alpha-amylase) soln. - No 120 L, Novo Laboratories, Inc, Wilton, CT 06897 or No A-0164, Sigma. Chemical Co, St Louis, MO 63178. Store in refrigerator. (See h below.)

3.6 Protease - No P-3910, Sigma Chemical Co, St Louis, MO 63178. Keep the dry enzyme refrigerated after each use. (See h below.)

3.7 Amyloglucosidase - No A-9913, Sigma Chemical Co, St Louis, MO 6178. Keep refrigerated. (See h below.)

3.8 Alternatively a kit containing all three enzymes (pretested) is available from Novo BioLabs (Fibre Zym Kit) Wilton, CT 06897 and Sigma Chemical Co. Cat#TDF-100.

3.9 Sodium hydroxide soln. - 0.275N. Dissolve 11.00 g NaOH ACS in ca 700 ml H_2O in 1 L volumetric flask. Dilute to volumetric with H_2O.

3.10 Hydrochloric acid soln. - 0.350N. Dilute a stock solution with known titer, e.g. 350 ml 1M HC1 to one litre with distilled H_20.

3.11 Celite C-211 - Acid washed incinerated, Fisher Scientific Co, Pittsburgh, PA 15219.

4. Enzyme Purity

To ensure the absence of undesirable enzymatic activity in the enzymes used in this procedure, run materials listed in the table through the entire procedure each time the enzymes lot is changed, or at maximum interval of 6 months to ensure that enzymes have not been degraded.

Test Sample	Activity Tested	Sample Wt., g	Expected Recovery %
Citrus Pectin	pectinase	0.1	95-100
Stractan (Larch Gum)	hemicellulase	0.1	95-100
Wheat Starch	amylase	1.0	0-1
Corn Starch	amylase	1.0	0-2
Casein	protease	0.3	0-2
β-Glucan (Barley Gum)[a]	β-glucanase	0.1	95-100

[a](Sigma Chemical Co St Louis, MO 63178)

5. Sample Preparation

Determine insoluble and soluble dietary fibre on dry samples, without pretreatment whenever possible. Mill dry foods with particles >0.5mm to 0.3-0.5mm mesh. Homogenise and freeze-dry wet foods before milling. If high fat content (>10%) prevents proper milling, defat with petroleum ether (3 times with 25ml portions/g sample) before milling. Determine residual moisture in milled samples by drying overnight in 70 °C vacuum oven or for 5 hours in 105 °C air oven. Record losses of weight due to fat and/or H_2O removal and report final % dietary fibre on a dry matter basis.

6. Determination

6.1 Run blank through entire procedure along with samples to measure any contribution from reagents to residue.

6.2 Weigh duplicate 1 g samples, accurate to 0.1 mg, into 400 ml tall-form beakers. Sample weights should not differ by more than 20 mg. Add 50 ml *p*H 6.0 phosphate buffer to each beaker. Check *p*H with pH meter. Adjust to *p*H 6.0 + 0.2 with 0.275M NaOH if necessary.

6.3 Add 0.1 ml Termamyl soln.

6.4 Cover beaker with Al foil and place in boiling H_2O bath 15 min. Shake gently at 5 min intervals. Increase incubation time when number of beakers in boiling H_2O bath makes it difficult for beaker contents to reach temp of 100 °C. Use thermometer to ensure 15 min at 100 °C. Thirty min should be sufficient.

6.5 Cool solutions to room temperature. Adjust to *p*H 7.5 + 0.2 by adding 10 mL 0.275M NaOH soln.

6.6 Add 5 mg protease. (Protease sticks to spatula, so it may be preferable to prepare the enzyme soln. (50 mg in 1 ml phosphate buffer) just before use and pipet 0.1 ml to each sample).

6.7 Cover beaker with Aluminium foil. Incubate 30 min at 60 °C with continuous agitation.

6.8 Cool. Add 10 ml of 0.350M hydrochloric acid solution. Measure *p*H and add acid dropwise if necessary. Final *p*H should be 4.0-4.6.

6.9 Add 0.3 ml of amyloglucosidase, cover with Al foil and incubate at 60 °C for 30 min with continuous agitation.

A. Soluble Dietary Fibre

6.10 Tare crucible containing Celite to the nearest 0.1 mg. Then wet and redistribute the bed of Celite in the crucible using a stream of distilled water from a wash bottle. Apply suction to the crucible to draw the Celite onto the fritted glass as an even mat.

6.11 Filter the enzyme mixture from step (i) through the crucible into a pre-tared suction flask.

6.12 Wash residue twice with 10 ml of distilled water. Save the filtrate and water washings for determination of soluble dietary fibre.

6.13 Wash residue twice with 10 ml each of 95% EtOH and twice with 10 mL each acetone. With some samples, a gum is formed, trapping the liquid. If the surface film that develops after the addition of the sample to the Celite is broken with a spatula, filtration is improved. Long filtration times can be avoided by careful intermittent suction throughout the filtration. Normal suction can be applied at washing. Back-bubbling with air, if available, is another way of speeding filtration.

6.14 Dry crucible containing residue overnight in a 70 °C vacuum oven or a 105 °C air oven.

6.15 Cool in desiccator and weigh crucible, Celite, and residue to nearest 0.1 mg. Subtract crucible and Celite weight to determine weight of residue.

6.16 Analyse the residue from one sample of the set of duplicates for protein. Protein is probably most easily analysed by carefully scraping the Celite and the fibre mat onto a suitable piece of filter paper which can be folded shut and analysed for protein. A piece of filter paper should be analysed to assure that it will not affect the protein value obtained. To determine the nitrogen content use the Kjeldahl analysis as specified in "Official Methods of Analysis" of the AOAC 47.021-47.023.

Use nitrogen × 6.25 for the protein factor in all cases.

6.17 Incinerate second residue sample of the duplicate for 5 hours at 525 °C. Cool in desiccator and weigh crucible to nearest 0.1 mg. Subtract crucible and Celite weight to determine ash. Negative ash weight might occur as a consequence of insufficient drying of Celite or losses, especially at IDF filtration. Replace crucible if ash is <-3 to -5 mg. Always use actual ash weights: positive or negative - in the calculation.

B. Soluble Dietary Fibre

6.18 Adjust the weight of the combined filtrate and water washing from step (1) of the insoluble fibre procedure to 100 g with distilled water, (the density of the filtrate is close to one.)

6.19 Transfer the solution to a beaker or Erlenmyer. Add 4 volumes (400 mL) of 95% EtOH preheated to 60 °C. Rinse the suction flask with part of the EtOH.

6.20 Allow precipitate to form at room temperature for 60 min.

6.21 Tare crucible containing Celite to nearest 0.1 mg then wet and redistribute the bed of Celite in the crucible using a stream of 78% EtOH from a wash bottle. Suction is then applied to the crucible to draw the Celite onto the fritted glass as an even mat. When fibre is filtered, i.e. step (n), the Celite effectively separates the fibre from the fritted glass of the crucible allowing for easy removal of the crucible contents.

6.22 Filter enzyme digest from step (l) through crucible.

6.23 Wash residue successively with three 20 ml portions of 78% ethanol, two 10 ml portions of 95% ethanol, and two 10 ml portions of acetone. With some samples, a gum is formed, trapping the liquid. If the surface film that develops after the addition of the sample to the Celite is broken with a spatula, filtration is improved. Long filtration times can be avoided by careful intermittent suction throughout the filtration. Normal suction can be applied at washing Back-bubbling with air, if available, can be used to speed filtration.

6.24 Proceed with step (n) through (q) of insoluble dietary fibre method.

7. Calculation

Calculations have been simplified by the use of new data sheets and equations. Blanks and Samples and the new equations for calculation of dietary fibre have rectified all of the problems associated with the calculations. (Figure 1)

BLANK DATA SHEET

	a		b		c		d	
Crucible + Celite + Tare weight (mg)								
Crucible + Celite + Residue weight (mg)								
Residue weight (mg)	R_1	R_2	R_1	R_2	R_1	R_2	R_1	R_2
Protein (mg)								
Crucible + Celite + Ash weight (mg)								
Ash weight (mg)								
Blanks (mg)								
Mean Blank (a+b+c+d) A (mg) B								

BLANKS

$$\text{Blanks (mg)} = \frac{R_1+R_2}{2} - P - A$$

PROSKY DIETARY FIBRE METHOD SAMPLE DATA SHEET

	Sample				Sample			
	1		2		1		2	
1 Sample weight (mg)	m_1	m_2	m_1	m_2	m_1	m_2	m_1	m_2
2 Crucible + Celite Tare weight (mg)								
3 Crucible + Celite + Residue weight (mg)								
4 Residue weight (mg)	R_1	R_2	R_1	R_2	R_1	R_2	R_1	R_2
5 Protein (mg)								
6 Crucible + Celite + Ash weight (mg)								
7 Ash weight (mg) A								
8 Mean Blank (mg) B								
9 Dietary Fibre (%)								

$$\text{Dietary Fibre (\%)} = \frac{\frac{R_1+R_2}{2} - P - A - B}{\frac{m_1+m_2}{2}} \times 100$$

TABLE II

Englyst Colorimetric Determination :Sample A - Coconut

Laboratory	Soluble Dietary fibre (i)	(ii)	Insoluble Dietary fibre (i)	(ii)	Total Dietary fibre (i)	(ii)
2	0.1	0.2	11.5	11.5	11.6	11.7
3	0.6	0.8	13.8	13.6	14.4	14.4
4	1.6		10.8		12.4	
5	0.5	1.5	15.1	14.5	15.6	16.0
6	2.2	2.9	10.2	9.6	12.3	12.5
7	3.1		8.2		11.3	
8	0.6	1.3	11.1	11.1	11.7	12.4
9	1.0	1.0	12.1	12.1	13.1	13.1
10	0.7	0.2	14.2	14.1	14.9	14.3
11	1.7		8.9		10.5	
12	1.7	2.1	12.6	12.4	14.3	14.5
16	0.3	0.5	9.0	9.6	9.3	10.1
17	3.6	2.3	10.4	10.9	14.0	13.3
18	0.1		11.3		11.4	
20	1.0	1.1	11.5	11.3	12.4	12.4
21	1.5	1.1	13.2	12.8	14.7	13.9
24	1.9	1.1	11.6	12.3	13.5	13.4
25	0.3		12.0		12.3	
26	6.4[b]	0.1[b]	6.8 [b]	12.6[b]	13.1	12.6
28	1.7	1.5	11.4	11.7	13.1	13.2
29	2.0	2.0	10.6	11.3	12.6	13.3
32	0.0	0.2	8.5	8.1	8.5	8.3
33	3.7	3.0	10.2	11.3	13.9	13.3
35	0.3		13.8		14.1	
36	2.5	3.0	11.9	11.4	14.4	14.8
37	0.6	0.7	13.3	13.0	13.8	13.7

Mean	1.39	11.71	13.06
r	1.09	0.95	0.91
SD_r	0.39	0.34	0.32
RSD_r (%)	28.19	2.91	2.48
R	2.82	4.62	4.83
SD_R	1.01	1.64	1.73
RSD_R (%)	72.60	14.09	13.22

For Key to Tables II-XXXVII: See Table XXXVIII

TABLE III

Englyst GLC Determination: Sample A - Coconut

Laboratory	Soluble Dietary fibre (i)	(ii)	Insoluble Dietary fibre (i)	(ii)	Total Dietary fibre (i)	(ii)
2	1.8	1.3	11.6	11.4	13.4	12.7
3	0.5	0.5	11.0	10.5	11.5	11.0
4	2.3		10.4		12.7	
5	0.0	0.7	12.2	12.1	12.1	12.8
7	1.3		12.7		13.7	
8	0.3	0.8	11.5	10.4	11.8	11.2
11	3.6		10.1		13.7	
12	1.1	0.8	11.0	11.6	12.1	11.9
16	0.8	2.0	7.9	9.7	8.7	11.7
17	1.5	2.3	9.3	9.8	10.8	12.1
20	1.2	0.9	11.4	11.9	12.6	12.7
21	2.5	2.0	10.8	11.0	13.3	13.0
22	1.5	0.6	11.6	12.1	13.1	12.7
24	0.0	0.2	13.2	12.0	13.2	12.2
25	1.2		10.9		12.1	
28	1.4	1.1	11.5	11.6	12.9	12.7
29	0.0	0.0	12.5	12.5	12.5	12.5
33	2.8	2.0	9.9	10.0	12.7	12.0
36	1.9	3.8	9.9	11.7	11.8	15.5
37	1.1	1.3	11.2	11.1	12.3	12.4
Mean		1.21		11.12		12.31
r		1.46		1.61		2.61
SD_r		0.52		0.58		0.93
RSD_r (%)		43.19		5.18		7.57
R		2.54		3.10		3.03
SD_R		0.91		1.11		1.08
RSD_R (%)		75.05		9.95		8.79

TABLE IV

Prosky Determination: Sample A - Coconut

Laboratory	Soluble Dietary fibre (i)	Soluble Dietary fibre (ii)	Insoluble Dietary fibre (i)	Insoluble Dietary fibre (ii)	Total Dietary fibre (i)	Total Dietary fibre (ii)
7	0.0	0.0	12.2	13.1	12.2	13.1
9	0.1	0.3	31.6[c]	27.5[c]	31.8	27.8
10	0.6	0.4	14.7	16.2	15.3	16.6
17	1.0		14.5	13.8	15.5	13.8
20	0.0	0.2	9.6	10.2	9.6	10.4
24	0.6	0.7	12.6	13.2	13.2	13.9
25	1.5		12.2		13.7	
26	0.7	0.4	16.9	15.5	17.6	16.0
27	0.4	0.1	12.2	12.4	12.6	12.5
33	0.3	0.1	13.0	13.9	21.2	22.2
35	0.3		13.9		14.2	
Mean	0.31		13.38		16.41	
r	0.41		1.86		3.37	
SD_r	0.15		0.67		1.20	
RSD_r (%)	48.29		4.97		7.34	
R	0.71		5.64		17.00	
SD_R	0.25		2.02		6.07	
RSD_R (%)	83.13		15.07		37.00	

TABLE V

Englyst Colorimetric Determination: Sample B: Porridge

Laboratory	Soluble Dietary fibre (i)	(ii)	Insoluble Dietary fibre (i)	(ii)	Total Dietary fibre (i)	(ii)
1	4.1		3.7		7.8	
2	5.1	5.0	3.8	3.9	8.9	8.9
3	3.4	2.7	5.9	6.6	9.3	9.3
4	7.8		4.5		12.2	
5	3.7	5.0	5.0	4.4	8.7	9.4
6	3.8	4.0	3.9	4.1	7.7	8.0
7	2.9		3.1		6.0	
8	3.5	4.3	3.8	3.7	7.3	8.0
9	3.0	4.0	5.2[b]	3.7[b]	8.2	7.7
10	4.8	4.9	3.6	3.5	8.4	8.4
11	4.2		3.0		7.2	
12	4.4	5.4	3.8	3.2	8.2	8.6
13	4.1		3.6		7.7	
14	4.4	4.8	3.6	3.6	8.0	8.4
15	5.0	5.2	5.1	5.2	10.1	10.5
16	2.2	3.3	3.5	3.4	5.7[c]	6.7[c]
17	5.3	6.9	1.5	1.9	6.8[b]	8.8[b]
18	4.3		4.7		9.0	
20	5.1	5.7	4.4	4.3	9.5	10.0
21	4.5	4.6	4.2	4.1	8.7	8.7
23						8.0
24	4.5	4.8	3.4	3.2	7.9	
25	3.8		3.1		6.9	
26	5.4	3.2	7.3[c]	8.8[c]	12.7[c]	12.0[c]
28	4.1	4.2	4.1	4.0	8.1	8.2
29	4.2	4.2	4.4	3.6	8.0	8.4
31	4.3	4.5	3.7	3.8	8.1	8.2
32	5.1	5.0	3.8	3.9	8.9	8.9
33	4.7	5.0	3.7	3.5	8.3	8.5
34	4.6	4.3	4.2	3.9	8.5	8.5
35	4.9		5.4		10.3	
36	5.6	4.9	5.0	5.0	10.6	9.9
37	3.6	3.8	6.0	5.8	9.6	9.6
Mean		4.46		4.07		8.69
r		1.56		0.65		0.72
SD_r		0.56		0.23		0.26
RSD_r (%)		12.51		5.69		2.95
R		2.38		2.67		2.21
SD_R		0.85		0.95		0.79
RSD_R (%)		19.10		23.46		9.08

TABLE VI

Englyst GLC Determination - Sample B - Porridge

Laboratory	Soluble Dietary fibre (i)	(ii)	Insoluble Dietary fibre (i)	(ii)	Total Dietary fibre (i)	(ii)
2	4.9	4.9	4.1	3.8	8.9	8.7
3	3.4	3.8	3.5	3.7	6.9	7.5
4	7.7		2.9		10.6	
5	2.8	4.2	4.5	3.9	7.3	8.1
7	3.2		4.3		7.5	
8	3.0	3.7	3.4	3.4	6.4	7.1
11	2.6		2.9		5.5	
12	3.3	4.6	3.8	2.8	7.1	7.4
13	3.6		3.4		7.0	
14	3.7	3.4	3.4	3.6	7.1	7.0
16	1.9	3.4	3.0	3.1	4.9	6.5
17	3.9	4.4	2.5	2.8	6.4	7.2
20	4.5	4.0	2.7	3.0	7.2	7.0
21	3.4	3.9	4.5	3.8	7.9	7.7
22	3.8	3.5	3.8	3.5	7.5	7.0
24	3.2	3.0	2.7	2.9	5.9	5.9
25	3.7		2.8		6.5	
28	4.1	3.7	3.5	3.3	7.5	7.0
29	3.2.	3.2	4.1	3.9	7.3	7.1
33	3.9	3.8	3.1	3.1	7.0	6.9
36	4.7	4.5	3.1	3.1	7.8	7.6
37	2.5	2.9	5.8[c]	5.5[c]	8.4	8.4
Mean	3.68		3.42		7.22	
r	1.35		0.77		1.13	
SD_r	0.48		0.28		0.40	
RSD_r (%)	13.09		8.04		5.57	
R	1.94		1.46		2.27	
SD_R	0.69		0.52		0.81	
RSD_R (%)	18.78		15.25		11.24	

TABLE VII

Prosky Determination: Sample B - Porridge

Laboratory	Soluble Dietary fibre (i)	(ii)	Insoluble Dietary fibre (i)	(ii)	Total Dietary fibre (i)	(ii)
7	3.2	3.4	4.5	4.1	7.7	7.5
9	2.0	2.4	4.8	4.7	6.8	7.0
10	0.5	0.5	5.9	6.4	6.4	6.9
14	3.8		5.5	5.6	9.4	
17	0.1	0.1	5.4	5.9	5.5	6.0
18	3.6		5.6		9.2	
19					9.9	
20	1.8	1.8	5.8	5.9	7.6	7.6
24	1.4	1.0	5.0	6.3	6.4	7.3
25	3.2		7.2		10.4	
26	3.4[(b)]	1.6[(b)]	5.7	4.3	9.0[(b)]	5.9[(b)]
27	3.1	3.3	5.9	5.5	9.0	8.8
30					6.5	6.5
33	3.4	4.1	5.1	5.6	10.5	11.7
34	0.2	0.0	4.7	4.7	4.9	4.7
35	2.7		6.8		9.5	
Mean	1.79		5.33		7.27	
r	0.64		1.30		1.07	
SD_r	0.23		0.46		0.38	
RSD_r (%)	12.67		8.71		5.25	
R	3.99		1.84		4.93	
SD_R	1.42		0.66		1.76	
RSD_R (%)	79.32		12.32		24.34	

TABLE VIII

Englyst Colorimetric Determination: Sample C - Baked Beans

Laboratory	Soluble Dietary fibre (i)	(ii)	Insoluble Dietary fibre (i)	(ii)	Total Dietary fibre (i)	(ii)
1	7.9		5.6		13.6	
2	8.3	8.0	5.8	5.9	14.1	13.9
3	5.1	5.7	8.1	7.8	13.2	13.5
4	8.8		4.3		13.1	
5	6.4	8.1	7.4	6.7	13.8	14.8
6	5.7	6.9	6.4	5.7	12.1	12.6
7	5.3		6.4		11.7	
8	7.7	6.5	6.0	5.6	13.7	12.1
9	6.4	6.4	6.0	5.4	12.4	11.8
10	6.9	6.8	5.9	6.1	12.8	12.9
11	6.9		5.1		12.0	
12	6.7	7.8	6.7	6.1	13.4	13.9
13	7.6		5.7		13.3	
14	7.3	7.0	5.1	5.4	12.4	11.6
15	6.8	6.8	6.6	6.8	13.4	13.5
16	6.0	5.5	4.7	4.4	10.7	9.7
17	9.3	8.0	4.9	4.6	14.2	12.6
18	6.0		6.0		12.0	
20	8.1	8.6	7.0	6.8	15.1	15.4
21	7.7	7.6	6.9	6.6	14.6	14.2
24	6.9	6.8	5.8	5.5	12.7	12.3
25	9.0		4.8		13.8	
26	8.4	5.4	3.2[b]	6.9[b]	11.6	12.3
28	6.8	7.5	5.7	5.6	12.5	13.1
29	9.4	9.4	5.1	5.5	14.1	15.2
31	9.0	9.2	5.9	6.8	14.9	16.0
32	5.1[b]	9.1[b]	2.4[b]	0.1[b]	7.5	9.2
33	7.4	6.9	5.9	6.1	13.2	13.0
34	6.9	8.0	6.1	5.9	13.0	13.9
35	8.5		5.4		13.9	
36	7.5	7.5	8.0	8.9	15.5	14.4
37	7.2	7.2	6.5	6.4	13.7	13.6
Mean	7.29		6.16		13.13	
r	1.88		0.89		4.52	
SD_r	0.67		0.32		0.60	
RSD_r (%)	9.20		5.14		4.54	
R	3.00		2.65		4.52	
SD_R	1.07		0.95		1.62	
RSD_R (%)	14.67		15.38		12.30	

TABLE IX

Englyst GLC Determination: Sample C - Baked Beans

Laboratory	Soluble Dietary fibre (i)	(ii)	Insoluble Dietary fibre (i)	(ii)	Total Dietary fibre (i)	(ii)
2	8.7	8.1	6.3	6.3	15.0	14.4
3	5.6	6.2	5.8	5.6	11.4	11.8
4	7.8		4.4		12.2	
5	6.5	7.4	6.3	5.8	12.8	13.2
7	7.0		7.0		14.0	
8	7.1	6.9	5.9	5.5	13.0	12.4
11	8.5		4.8		13.3	
12	7.7	6.1	6.1	6.9	13.8	13.0
13	8.3		5.7		14.0	
14	7.1	6.8	5.5	5.0	12.6	11.9
16	6.2	6.0	4.6	4.3	10.8	10.3
17	8.0	6.9	4.8	4.8	12.8	11.6
20	7.3	7.6	5.7	5.7	13.0	13.3
21	6.9	7.5	6.2	5.8	13.1	13.3
22	7.7	7.2	5.5	5.7	13.2	12.9
24	6.4	6.0	4.5	4.3	10.7	10.5
25	7.6		4.7		12.3	
28	7.0	7.2	5.4	5.2	12.4	12.4
29	6.9	6.9	5.6	6.1	13.1	12.4
33	7.7	7.3	5.1	5.2	12.8	12.5
34	7.4		5.2		12.6	
36	4.3[c]	2.0 [c]	6.1	5.0	10.4[b]	7.0[b]
37	7.4	7.6	6.2	6.1	13.7	13.7
Mean	7.06		5.56		12.62	
r	1.27		0.86		1.07	
SD_r	0.45		0.31		0.38	
RSD_r (%)	6.42		5.51		3.04	
R	1.95		1.80		3.06	
SD_R	0.70		0.64		1.09	
RSD_R (%)	9.87		11.56		8.67	

TABLE X

Prosky Determination: Sample C - Baked Beans

Laboratory	Soluble Dietary fibre (i)	(ii)	Insoluble Dietary fibre (i)	(ii)	Total Dietary fibre (i)	(ii)
7	6.6	7.1	11.1	10.9	17.7	18.1
9	5.2	5.7	10.0	10.4	15.2	16.1
10	6.4	6.2	10.8	10.2	17.2	16.4
14	5.9		11.6	11.2	17.3	
17	5.8[b]	4.2[b]	11.3	11.7	17.2	15.9
18	6.3		13.9		20.2	
19					18.2	
20	5.4	5.6	11.7	11.7	17.0	17.3
21	6.9	7.5	6.2[c]	5.8[c]	13.1	13.3
24	6.8	6.5	11.3	11.8	18.1	18.3
25	6.6		13.9		20.5	
26	5.0	5.2	9.1[c]	6.1[c]	14.1	11.3
27	6.3	6.1	10.9	11.2	17.2	17.3
30					15.1	15.4
33	6.1	6.1	9.8	10.4	17.3	17.5
34	4.2	4.2	12.8[b]	9.1[b]	17.0	13.3
35	4.8		12.6		17.4	
Mean	5.96		11.00		16.10	
r	0.66		0.83		2.87	
SD_r	0.24		0.30		1.02	
RSD_r (%)	3.96		2.69		6.36	
R	2.57		1,79		5.34	
SD_R	0.92		0.64		1.91	
RSD_R (%)	15.39		5.80		11.85	

TABLE XI
Englyst Colorimetric Determination :Sample D - Cabbage

Laboratory	Soluble Dietary fibre (i)	(ii)	Insoluble Dietary fibre (i)	(ii)	Total Dietary fibre (i)	(ii)
1	10.1		12.1		22.2	
2	10.0	10.0	12.6	12.7	22.6	22.7
3	8.0	8.9	15.0	14.1	23.0	23.0
4	10.4		11.2		21.5	
5	10.2	9.4	17.3	17.0	27.5	26.4
6	8.6	10.0	13.3	13.2	22.0	23.2
7	6.3		13.1		19.4	
8	13.5	11.2	14.2	13.3	27.7	24.5
9	9.8	7.4	11.9	11.9	21.7	19.3
10	9.4	9.4	13.3	13.1	22.7	22.5
11	6.8		12.3		19.1	
12	10.6	11.9	13.4	13.3	24.0	25.2
13	12.3		13.9		26.2	
14	10.1	10.9	14.5	15.0	24.6	25.9
15	7.8	8.1	13.8	14.3	21.8	22.4
16	11.0	13.1	10.8	11.4	21.8	24.5
17	9.7	10.4	12.9	11.8	22.5	22.2
18	7.0		15.0		22.0	
20	11.3	12.0	14.8	14.0	26.1	26.0
21	8.4	9.0	15.7	15.1	24.1	24.1
24	11.3	8.9	15.4	15.4	26.7	24.3
25	11.6		14.1		25.7	
26	10.6	9.3	10.1	9.4	20.7	18.6
28	8.8	10.4	14.4	14.2	23.2	24.6
29	12.2	12.2	14.3	13.2	25.8	26.1
32	4.2	6.0	6.4[c]	6.2[c]	10.6[c]	12.2[c]
33	10.6	11.2	13.1	13.0	23.6	24.2
34	8.6	9.8	12.9	13.3	21.5	23.1
35	14.0		13.8		27.8	
36	7.7	11.9	16.4[b]	21.4[b]	24.1[b]	33.4[b]
37	11.5	11.7	14.7	14.8	26.7	26.4

Mean	9.93	13.61	23.80
r	3.07	1.16	2.89
SD_r	1.10	0.41	1.03
RSD_r (%)	11.04	3.05	4.34
R	5.05	4.59	5.92
SD_R	1.80	1.64	2.12
RSD_R (%)	18.16	12.05	8.89

TABLE XII
Englyst GLC Determination: Sample D - Cabbage

Laboratory	Soluble Dietary fibre (i)	Soluble Dietary fibre (ii)	Insoluble Dietary fibre (i)	Insoluble Dietary fibre (ii)	Total Dietary fibre (i)	Total Dietary fibre (ii)
2	12.9	15.4	14.0	13.4	26.8	28.8
3	6.3	6.9	12.1	11.1	18.4	18.0
4	14.8		8.4		23.2	
5	12.9	15.0	16.3	15.8	29.2	30.8
7	16.4		7.3		23.7	
8	13.8	13.4	12.9	13.2	26.7	26.6
11	16.4		12.0		28.4	
12	16.2	12.3	12.2	12.4	28.4	24.7
13	12.6		14.1		26.7	
14	13.1	12.5	13.3	14.4	26.3	26.8
16	11.6	11.3	9.6	9.8	21.2	21.1
17	11.9	10.8	9.9	11.0	21.8	21.8
20	10.5	13.5	11.9	10.8	22.4	24.3
21	12.3	13.1	13.5	13.1	25.8	26.2
22	13.2	13.1	13.0	12.8	26.2	25.9
24	14.1	10.4	13.6	14.2	27.7	24.6
25	12.3		12.1		24.3	
28	11.7	13.0	13.8	14.1	25.5	27.1
29	9.0	9.0	16.4	14.9	23.7	25.6
33	13.3	12.3	11.6	11.8	24.9	24.1
36	7.1	12.0	13.3	15.7	20.4[b]	27.7[b]
37	14.6	14.7	13.7	13.7	28.3	28.4
Mean	12.15		13.04		25.25	
r	4.24		1.80		3.17	
SD_r	1.52		0.64		1.13	
RSD_r (%)	12.47		4.94		4.48	
R	6.61		4.93		8.54	
SD_R	2.36		1.76		3.05	
RSD_R (%)	19.43		13.51		12.08	

TABLE XIII
Prosky Determination: Sample D - Cabbage

Laboratory	Soluble Dietary fibre (i)	Soluble Dietary fibre (ii)	Insoluble Dietary fibre (i)	Insoluble Dietary fibre (ii)	Total Dietary fibre (i)	Total Dietary fibre (ii)
7	6.7	6.8	19.4	18.7	26.1	25.5
9	4.6	3.1	19.7	21.0	24.3	24.2
10	6.4	4.9	21.0	22.4	27.4	27.3
14	4.1		28.1	26.4	31.4	
17	5.1	4.0	22.1	22.8	27.2	26.8
18	8.6		22.6		31.2	
19					29.2	
20	5.0	6.4	23.1	22.5	28.1	28.9
25	10.3		20.8		31.1	
26	7.5	6.9	21.0	19.5	28.5	26.4
27	4.8	4.6	23.5	22.9	28.3	27.4
30					23.5	22.9
33	7.5	6.1	21.4	21.7	30.0	28.7
34	9.6	8.9	19.3	19.3	28.9	32.0
35	2.9		26.5		29.4	
Mean	6.05		21.98		27.12	
r	2.14		3.13		2.66	
SD_r	0.76		1.12		0.95	
RSD_r (%)	12.64		5.09		3.50	
R	4.86		6.66		6.47	
SD_R	1.74		2.38		2.31	
RSD_R (%)	28.72		10.82		8.52	

TABLE XIV
Englyst Colorimetric Determination: Sample E - Mash Potato

Laboratory	Soluble Dietary fibre (i)	(ii)	Insoluble Dietary fibre (i)	(ii)	Total Dietary fibre (i)	(ii)
1	4.0		2.8		6.8	
2	3.0	3.8	2.5	2.7	5.5	6.5
3	3.6	3.7	5.9[c]	5.8[c]	9.5	9.5
4	4.2		2.4		6.5	
5	4.0	4.1	3.8	4.0	7.8	8.1
6	3.3	3.3	2.9	2.5	6.3	5.8
7	3.3		2.0		5.3	
8	3.8	4.2	2.8	3.3	6.6	7.5
9	3.5	3.6	2.9	3.0	6.3	6.6
10	4.0	4.3	2.8	2.3	6.8	6.6
11	3.7		2.4		6.1	
12	3.7	3.7	3.4	3.4	7.1	7.1
13	4.6		3.0		7.6	
14	3.7	4.1	2.6	2.9	6.2	7.0
16	4.0	4.6	2.9	2.4	6.9	7.0
17	3.5	4.4	3.3	2.1	6.8	6.5
18	3.7		2.0		5.7	
19	3.6		2.4		6.0	
20	4.1	4.4	3.2	3.4	7.3	7.8
21	3.3	3.6	5.0[c]	5.3[c]	8.3	8.9
24	3.6	3.7	2.5	2.3	6.0	6.1
25	4.5		2.4		6.9	
26	6.2[b]	4.0[b]	2.1	2.2	5.7[b]	8.3[b]
28	4.1	4.1	2.9	2.9	7.0	6.9
29	5.1[c]	5.1[c]	3.2	2.8	8.3	7.9
31	3.4	3.6	2.9	2.2	6.3	5.8
32	3.9	4.0	1.9	1.9	5.8	6.0
33	4.0	3.9	2.7	2.6	6.8	6.6
34	4.3	3.6	3.0	3.2	7.3	6.8
35	4.2		3.4		7.6	
36	3.9	3.9	4.4	4.4	8.4	8.4
37	4.1	4.1	4.1	4.2	8.1	8.3

Mean	3.85	2.94	7.12
r	0.75	0.79	0.89
SD_r	0.27	0.28	0.32
RSD_r (%)	6.94	9.57	4.46
R	0.97	1.80	2.81
SD_R	0.34	0.64	1.00
RSD_R (%)	8.96	21.94	14.08

TABLE XV
Englyst GLC Determination: Sample E - Mash Potato

Laboratory	Soluble Dietary fibre (i)	(ii)	Insoluble Dietary fibre (i)	(ii)	Total Dietary fibre (i)	(ii)
2	4.3	4.8	2.8	3.0	7.2	7.8
3	3.3	4.1	3.4	2.7	6.7	6.8
4	3.8		2.5		6.3	
5	3.8	4.8	2.9	2.5	6.7	7.3
7	4.5		3.1		7.6	
8	3.8	4.3	2.8	2.8	6.6	7.1
11	4.3		2.3		6.6	
12	3.9	3.6	3.0	3.2	6.9	6.8
13	4.3		2.9		7.2	
14	3.9	3.8	2.7	3.1	6.6	6.9
16	4.0	3.6	2.4	2.3	6.4	6.0
17	4.0	4.5	2.7	2.3	6.8	6.7
20	4.1	4.2	2.8	2.7	6.8	6.9
21	4.2	3.9	3.2	3.3	7.4	7.2
22	4.3	4.0	2.9	3.0	7.2	6.9
24	3.5	3.4	2.1	2.2	5.6	5.6
25	3.8		2.3		6.1	
28	4.2	4.0	2.8	3.0	7.0	7.0
29	4.2	4.2	3.0	1.8	6.5	6.7
33	4.5	4.2	2.3	2.3	6.5[(b)]	8.1[(b)]
36	4.0	3.7	3.4	3.5	7.4	7.2
37	3.9	3.9	3.3	4.0	7.3	7.9
Mean	4.03		2.83		6.87	
r	0.84		0.84		0.67	
SD_r	0.30		0.30		0.24	
RSD_r (%)	7.46		10.64		3.48	
R	0.97		1.29		1.44	
SD_R	0.35		0.46		0.51	
RSD_R (%)	8.64		16.29		7.49	

TABLE XVI
Prosky Determination: Sample E - mash potato.

Laboratory	Soluble Dietary fibre (i)	(ii)	Insoluble Dietary fibre (i)	(ii)	Total Dietary fibre (i)	(ii)
7	2.2	2.2	6.1	6.1	8.3	8.3
9	1.8	1.8	5.7	5.4	7.5	7.3
10	1.6	2.2	5.6	5.8	7.2	8.0
14	1.6		6.4	6.5	8.1	
17	1.0	1.2	5.9	5.9	6.9	7.0
18	2.9		6.3		9.2	
19					7.6	
20	2.3	2.0	5.4	6.1	7.7	8.1
24	2.1	2.1	5.3	5.8	7.4	7.9
25	2.4		5.4		7.8	
26	2.2	2.5	4.9	4.0	7.0	6.5
27	2.5	2.3	5.8	5.3	8.3	7.6
30					6.9	6.7
33	2.7	2.6	4.2	5.8	8.1	8.0
34	3.8	2.6	6.2	5.5	10.0	8.1
35	3.2		5.4		8.6	
Mean	2.19		5.62		7.67	
r	0.90		1.33		1.40	
SD_r	0.32		0.48		0.50	
RSD_r (%)	14.72		8.47		6.52	
R	1.67		1.77		2.17	
SD_R	0.60		0.63		0.78	
RSD_R (%)	27.29		11.21		10.12	

TABLE XVII
Englyst Colorimetric Determination: Sample F - Banana

Laboratory	Soluble Dietary fibre (i)	(ii)	Insoluble Dietary fibre (i)	(ii)	Total Dietary fibre (i)	(ii)
2	2.2	2.9	1.9	1.8	4.1	4.6
3	1.8	2.1	6.4	5.7	8.2	7.8
4	2.4		2.2		4.6	
5	2.7	2.9	3.0	2.8	5.7	5.7
6	3.5	3.2	3.2	3.5	6.8	6.7
7	2.7		1.0		3.7	
8	2.4	2.0	2.3	2.9	4.7	4.9
9	2.6	2.5	2.3	3.0	5.0	5.4
10	2.8	2.9	2.3	1.9	5.1	4.8
11	1.8		1.7		3.5	
12	1.9	2.2	3.0	2.6	4.9	4.8
15	1.7	2.2	3.6	3.6	5.3	5.8
16	2.5	2.8	2.4	2.2	4.9	5.0
17	2.2	3.9	2.1	1.0	4.3	4.9
18	2.6		1.8		4.4	
20	3.5	2.5	2.3	2.6	5.8	5.1
21	3.2	3.6	2.5	2.8	5.7	6.4
24	2.6	2.6	1.6	1.6	4.2	4.2
25	1.7		2.8		4.5	
26	4.0	1.6	2.2	1.5	6.2[b]	3.1[b]
28	2.7	3.3	2.1	2.5	4.8	5.7
29	3.2	3.2	3.9	2.9	6.5	6.7
32	3.6	2.5	1.8	2.0	5.4	4.5
33	3.0	3.3	2.1	2.0	5.0	5.3
35	1.9		2.7		4.6	
36	1.9	2.9	3.0	3.1	4.9	6.0
37	2.9	2.8	2.3	2.3	5.2	5.0

Mean	2.73	2.46	5.40
r	1.60	0.93	1.02
SD_r	0.57	0.33	0.36
RSD_r (%)	20.97	13.57	6.76
R	1.66	1.79	2.61
SD_R	0.59	0.64	0.93
RSD_R (%)	21.74	26.05	17.27

TABLE XVIII
Englyst GLC Determination: Sample F - Banana

Laboratory	Soluble Dietary fibre (i)	(ii)	Insoluble Dietary fibre (i)	(ii)	Total Dietary fibre (i)	(ii)
2	2.7	3.5	1.8	1.9	4.6	5.4
3	2.1	1.9	1.5	1.8	3.6	3.7
4	2.7		1.9		4.6	
5	3.4	3.7	2.2	2.3	5.6	6.0
7	3.0		1.9		4.9	
8	2.6	2.7	1.8	1.5	4.4	4.2
11	2.6		1.8		4.4	
12	2.5	3.3	2.1	1.9	4.6	5.2
16	2.5	2.8	2.0	2.0	4.7	5.0
17	2.6	3.4	2.0	1.6	4.6	5.0
20	2.4	2.6	1.7	1.6	4.1	4.3
21	2.7	2.5	2.0	2.0	4.7	4.5
22	2.9	2.7	1.9	2.0	4.8	4.6
24	3.0	3.1	1.0	1.2	4.1	
25	3.5		1.1		4.6	
28	3.0	2.9	1.9	1.8	4.9	4.7
29	2.1	2.1	2.7	1.8	4.4	4.3
33	3.2	3.2	1.9	1.5	5.0	4.7
36	1.1[b]	3.6[b]	2.9[b]	1.1[b]	4.0	4.7
37	4.3	3.9	2.1	2.5	6.4	6.4

Mean	2.88	1.87	4.62
r	0.80	0.65	0.76
SD_r	0.29	0.23	0.27
RSD_r (%)	9.93	12.37	5.89
R	1.57	0.97	1.48
SD_R	0.56	0.35	0.53
RSD_R (%)	19.55	18.54	11.42

TABLE XIX
Prosky Determination: Sample F - Banana

Laboratory	Soluble Dietary fibre (i)	Soluble Dietary fibre (ii)	Insoluble Dietary fibre (i)	Insoluble Dietary fibre (ii)	Total Dietary fibre (i)	Total Dietary fibre (ii)
7	0.0	0.7	3.7	3.6	3.7	4.3
9	1.9	1.8	6.7	5.7	8.6	7.5
10	2.3	2.5	6.1	7.0	8.4	9.5
17	1.4	1.6	5.3	5.5	6.7	7.1
18	2.9		5.7		8.6	
20	1.4	2.2	5.4	5.1	6.8	7.3
22	7.7[c]	7.2[c]	5.5	5.7	13.2	12.9
24	0.7		5.6		6.3	
25	2.8		6.9		9.7	
26	2.7	2.0	5.7	4.2	8.5	6.1
27	2.7	2.3	5.2	5.9	7.9	8.2
33	3.0	2.6	4.9	5.1	8.4	8.0
35	0.8		6.7		7.5	
Mean	1.94		5.35		7.95	
r	1.00		1.44		2.01	
SD_r	0.36		0.51		0.72	
RSD_r (%)	18.33		9.62		9.04	
R	2.28		2.53		6.79	
SD_R	0.81		0.90		30.52	
RSD_R (%)	41.82		16.86		2.43	

TABLE XX
Englyst Colorimetric Determination: Sample G - Apples

Laboratory	Soluble Dietary fibre (i)	(ii)	Insoluble Dietary fibre (i)	(ii)	Total Dietary fibre (i)	(ii)
2	4.9	3.8	6.0	6.0	10.9	9.7
3	2.8		8.0		10.8	
4	4.4		5.4		10.3	
5	4.4	4.0	9.4	9.8	13.8	13.8
6	5.2	6.0	5.8	6.2	11.0	12.3
7	3.4		4.5		7.9	
8	4.5	5.0	7.6	7.1	12.1	12.1
9	3.0	3.6	6.0	5.8	9.0	9.4
10	3.5	4.0	6.7	6.1	10.2	10.1
11	5.3		6.1		11.4	
12	2.8	4.1	7.6	6.9	10.4	11.0
15	2.4	2.2	7.5	8.1	9.9	10.3
16	5.7	5.4	5.6	5.9	11.3	11.3
17	5.6	4.9	4.7	5.3	10.3	10.2
18	4.3		6.8		10.1	
20	3.9	4.6	6.6	6.5	10.5	11.1
21	3.8	4.0	7.7	8.1	12.1	11.5
24	3.8	3.9	6.3	6.2	10.1	10.1
26	1.5	1.1	5.6	5.2	7.1	6.2
28	4.0	5.3	7.0	6.0	11.0	11.4
29	5.3	5.3	6.4	6.7	11.8	11.0
32	5.0	2.1	0.3[b]	3.4[b]	5.3	5.5
33	4.9	3.5	6.8	7.0	11.6	10.5
36	4.6	7.0	10.2	10.0	14.8	17.0
37	3.3	3.4	8.5	8.3	11.9	11.7

Mean	4.13	6.93	10.78
r	2.16	0.89	1.55
SD_r	0.77	0.32	0.55
RSD_r (%)	18.63	4.58	5.14
R	3.50	3.83	6.23
SD_R	1.25	1.37	2.23
RSD_R (%)	30.24	19.75	20.64

TABLE XXI
Englyst GLC Determination: Sample G - Apples.

Laboratory	Soluble Dietary fibre (i)	(ii)	Insoluble Dietary fibre (i)	(ii)	Total Dietary fibre (i)	(ii)
2	5.9	5.6	7.3	7.5	13.2	13.0
4	4.7		6.3		11.0	
5	6.5	7.1	8.4	7.3	14.9	14.4
7	4.3		5.3		9.6	
8	6.0	4.8	6.7	7.0	12.7	11.8
11	6.2		7.1		13.3	
12	5.0	5.0	5.7	6.4	10.7	11.4
16	5.6	5.5	5.5	5.5	11.1	11.0
17	5.5	5.0	4.6	5.6	10.1	10.5
20	5.4	4.4	5.4	6.0	10.8	10.4
21	5.4	5.1	7.0	7.0	12.4	12.1
22	5.7	5.2	6.2	6.6	11.9	11.8
24	4.1	5.3	5.5	5.2	9.6	10.5
28	5.4	6.2	6.6	5.9	12.1	12.1
29	4.5	4.5	6.8	7.0	11.8	10.9
33	5.5	5.3	6.3	6.4	11.9	11.8
36	3.6	3.5	6.7	6.1	10.3	9.6
37	5.8	5.9	7.9	7.4	13.7	13.3
Mean	5.28		6.45		11.73	
r	1.21		1.10		1.06	
SD_r	0.43		0.39		0.38	
RSD_r (%)	8.21		6.06		3.23	
R	2.21		2.45		3.79	
SD_R	0.79		0.88		1.35	
RSD_R (%)	14.97		13.59		11.54	

TABLE XXII
Prosky Determination: Sample G - Apples

Laboratory	Soluble Dietary fibre (i)	(ii)	Insoluble Dietary fibre (i)	(ii)	Total Dietary fibre (i)	(ii)
7	3.1	4.2	8.2	8.3	11.3	12.5
9	2.4	2.9	8.3	8.9	10.7	11.8
10	4.8	4.7	8.2	7.6	13.0	12.3
17	0.0		6.5		6.5	
18	4.1		9.3		13.4	
20	3.7	3.6	8.7	9.0	12.3	12.6
26	2.9		8.2		11.0	
27	3.7	3.8	8.8	8.8	12.5	12.7
33	4.2	4.2	8.1	8.1	13.9	12.7
Mean	3.78		8.42		12.36	
r	0.99		0.73		1.75	
SD_r	0.35		0.26		0.63	
RSD_r (%)	9.33		3.11		5.07	
R	2.07		1.21		2.33	
SD_R	19.61		0.43		0.83	
RSD_R (%)	0.74		5.14		6.73	

TABLE XXIII
Englyst Colorimetric Determination: Sample H - Bread

Laboratory	Soluble Dietary fibre (i)	(ii)	Insoluble Dietary fibre (i)	(ii)	Total Dietary fibre (i)	(ii)
1	3.5		3.0		6.5	
2	3.2	3.2	2.8	2.9	6.1	6.1
4	3.3		3.0		6.3	
5	3.3	3.2	3.6	4.0	6.9	7.2
6	4.7	3.5	3.1	4.4	7.8	7.9
7	2.7		2.5		5.2	
8	3.2	2.9	3.2	3.2	6.4	6.1
9	3.0	2.8	2.9	3.1	5.9	5.8
10	3.7	3.1	3.0	3.8	6.7	6.9
11	2.7		2.8		5.5	
12	3.4	2.8	3.2	3.1	6.6	5.9
13	2.5		3.3		5.8	
14	3.1	3.7	3.1	2.9	6.3	6.6
15	3.7	3.4	4.4[c]	5.1[c]	8.0	8.5
16	4.2	2.4	2.9	3.7	7.1	6.1
17	3.4	4.8	2.6	1.7	6.0	6.5
18	3.2		3.3		6.5	
20	3.9	4.1	3.1	2.8	6.9	6.9
21	3.4	3.6	3.6	3.3	7.0	6.9
24	1.4[c]	2.1[c]	3.5	3.7	4.9	5.8
25	2.8		2.6		5.4	
26	3.4	2.9	2.7	3.3	6.1	6.1
28	3.4	3.0	3.0	3.3	6.5	6.3
29	3.8	3.8	3.0	3.3	7.1	6.7
31	3.5	3.2	3.7	3.5	7.2	6.7
32	2.4	2.6	3.1	2.8	5.5	5.4
33	3.3	3.1	3.4	3.5	6.7	6.6
34	3.6	3.7	3.2	3.1	6.8	6.8
35	4.5		2.8		7.3	
36	4.6[c]	5.5[c]	5.5[c]	5.3[c]	10.0[c]	10.8[c]
37	3.7	3.4	3.3	3.2	7.0	6.5
Mean	3.39		3.18		6.57	
r	1.28		0.97		0.83	
SD_r	0.46		0.35		0.30	
RSD_r (%)	13.42		10.89		4.51	
R	1.43		1.23		1.98	
SD_R	0.51		0.44		0.71	
RSD_R (%)	15.00		13.76		10.75	

TABLE XXIV
Englyst GLC Determination: Sample H - Bread

Laboratory	Soluble Dietary fibre (i)	(ii)	Insoluble Dietary fibre (i)	(ii)	Total Dietary fibre (i)	(ii)
2	3.2	3.1	3.2	3.5	6.4	6.6
4	3.4		1.8		5.2	
5	3.4	3.1	3.2	3.0	6.6	6.1
7	2.7		2.5		5.2	
8	2.4	2.6	3.2	2.6	5.6	5.2
9	3.0		2.9		5.9	
11	2.3		2.7		5.0	
12	2.6	2.3	2.7	2.8	5.3	5.1
13	2.7		2.7		5.4	
14	2.6	3.2	2.8	2.7	5.5	5.9
16	3.2	2.6	2.0	2.3	5.2	4.9
17	2.8[b]	4.6[b]	2.8	2.6	5.5[b]	7.3[b]
20	3.7	3.9	2.0	2.4	5.7	6.3
21	3.3	3.3	3.0	2.8	6.3	6.1
22	3.1	2.7	2.9	3.0	6.1	5.7
24	1.5	1.2	2.4	2.4	3.9	3.6
25	2.7		2.3		5.0	
28	2.7	2.9	2.7	2.7	5.4	5.6
29	2.8	2.8	3.0	3.0	5.8	5.8
33	2.6	2.5	3.0	2.8	5.6	5.4
36	3.2[b]	9.1[b]	3.0[b]	0.1[b]	6.2[b]	9.2[b]
37	2.9	2.7	2.8	2.8	5.8	5.5
Mean	2.82		2.77		5.61	
r	0.61		0.48		0.66	
SD_r	0.22		0.17		0.24	
RSD_r (%)	7.73		6.22		4.21	
R	1.61		0.96		1.96	
SD_R	0.57		0.34		0.70	
RSD_R (%)	20.38		12.38		12.46	

TABLE XXV
Prosky Determination: Sample H - Bread

Laboratory	Soluble Dietary fibre (i)	(ii)	Insoluble Dietary fibre (i)	(ii)	Total Dietary fibre (i)	(ii)
7	1.8	3.0	4.8	3.8	6.6	6.8
9	1.7	2.0	4.1	3.5	5.9	5.5
10	1.0	2.0	5.2	5.2	6.2	7.2
14	2.2		4.2	3.9	6.3	
17	3.4	3.5	4.8	4.5	8.2	8.0
18	2.6		6.1		8.7	
19					7.6	
20	1.9	1.5	5.0	5.1	6.9	6.6
24	2.0		5.3		7.3	
25	2.2		5.6		7.8	
26	0.6		2.7		3.3	
27	2.4	2.5	5.3	4.8	7.7	7.3
30					5.2	5.0
33	2.5	2.5	3.6	3.6	8.9	8.1
34	3.0	4.0	2.8	3.0	5.8	7.0
35	3.4		4.0		7.4	
Mean	2.42		4.29		6.83	
r	1.35		0.90		1.25	
SD_r	0.48		0.32		0.45	
RSD_r (%)	19.91		7.45		6.56	
R	2.30		2.26		3.15	
SD_R	0.82		0.81		1.13	
RSD_R (%)	34.01		18.85		16.50	

TABLE XXVI
Englyst Colorimetric Determination: Sample I+P - White bread

Laboratory	Soluble Dietary fibre (i)	(ii)	Insoluble Dietary fibre (i)	(ii)	Total Dietary fibre (i)	(ii)
1	1.4	2.2	2.4	1.7	3.7	3.8
2	1.6	2.0	1.6	1.4	3.2	3.4
3	1.6	1.7	4.5[(c)]	4.0[(c)]	6.1	5.7
4	1.2	2.3	2.5	1.7	3.6	4.0
5	3.0	2.3	2.8	2.3	5.8	4.6
6	1.8		2.2		4.0	
7	3.5	1.3	1.3	1.4	4.8	2.7
8	1.8	1.6	2.1	1.9	3.9	3.7
9	2.1	2.1	1.6	2.0	3.7	4.1
10	2.5	2.5	1.6	1.7	4.1	4.2
11	1.9	1.8	1.4	1.4	3.3	3.2
12	2.5	1.9	1.5	1.2	4.0	3.1
13	2.1	2.0	1.8	1.8	3.9	3.8
14	1.9	2.4	1.8	1.5	3.7	3.8
15	2.7	2.3	2.9	2.7	5.6	4.9
16	2.1	2.2	1.7	1.8	3.8	4.0
17	1.4	1.9	1.7	2.4	3.1	4.3
18	2.6	2.1	1.9	1.9	4.5	4.0
20	2.2	2.6	3.1	2.6	5.3	5.2
21	2.3	2.2	2.0	2.0	4.3	4.2
24	1.2	2.2	2.1	t	3.3	2.2
25	1.3	1.2	2.1	2.1	3.4	3.3
26	0.8	1.3	2.9	2.2	3. 6	3.5
28	2.4	2.2	1.7	2.0	4.2	4.2
29	2.8	2.9	1.5	2.3	4.3	5.2
31	3.5	2.5	1.8	1.5	5.3	4.0
32	0.9	2.9	2.5	1.6	3.4	4.5
33	2.3	2.3	2.1	2.1	4.5	4.5
34	4.1	2.2	2.3	2.2	6.4	4.4
35	1.2	1.4	1.9	2.8	3.1	4.2
36	1.8	4.3	3.4	1.8	5.2	6.1
37	1.6	1.9	2.0	2.1	3.7	4.1
Mean	2.11		2.00		4.16	
r	1.78		1.05		1.62	
SD_r	0.64		0.37		0.58	
RSD_r (%)	30.15		18.71		13.88	
R	1.92		1.35		2.42	
SD_R	0.69		0.48		0.87	
RSD_R (%)	32.52		24.13		20.83	

TABLE XXVII
Englyst GLC Determination: Sample I+P = Bread

Laboratory	Soluble Dietary fibre (i)	(ii)	Insoluble Dietary fibre (i)	(ii)	Total Dietary fibre (i)	(ii)
2	1.7	1.4	2.1	2.1	3.8	3.5
3	3.7	1.4	1.8	1.8	5.5[b]	3.2[b]
4	3.4	1.5	1.0	1.7	4.4	3.3
5	2.4	2.0	2.0	1.9	4.4	3.9
7	2.4	1.6	1.1	1.9	3.5	3.5
8	1.6	1.5	1.6	1.5	3.2	3.0
11	1.7	1.1	1.6	1.6	3.3	2.7
12	2.0	1.6	1.3	1.3	3.3	2.9
13	1.6	1.8	1.5	1.4	3.1	3.2
14	1.6	2.1	1.8	1.5	3.4	3.6
16	1.9	1.7	1.1	1.5	3.0	3.2
17	2.3	1.5	1.5	1.6	3.7	3.1
20	2.1	2.2	1.3	1.8	3.5	4.0
21	2.2	2.1	1.8	1.7	4.0	3.8
22	1.8	1.8	1.7	1.6	3.5	3.4
24	1.1	2.0	1.1	1.1	2.2	3.1
25	1.8	1.1	1.2	1.5	3.0	2.6
28	2.1	2.0	1.6	1.6	3.6	3.5
29	1.7	1.7	1.6	2.0	3.3	3.7
32	1.4	1.2	1.7	2.7	3.1	3.9
33	1.9	1.9	1.6	1.6	3.5	3.5
36	1.9[c]	4.6[c]	2.0[b]	0.0[b]	3.9	4.6
37	1.3	1.6	1.6	2.0	2.9	3.5
Mean	1.83		1.61		3.43	
r	1.50		0.74		0.99	
SD_r	0.54		0.27		0.35	
RSD_r (%)	29.38		16.45		10.29	
R	1.50		0.91		1.33	
SD_R	0.50		0.32		0.47	
RSD_R (%)	27.62		20.11		13.83	

TABLE XXVIII
Prosky Determination: Sample I+P = Bread

Laboratory	Soluble Dietary fibre (i)	Soluble Dietary fibre (ii)	Insoluble Dietary fibre (i)	Insoluble Dietary fibre (ii)	Total Dietary fibre (i)	Total Dietary fibre (ii)
7	1.0	0.3	2.2	2.0	3.2	2.3
9	1.1	1.4	2.4	2.3	3.5	3.7
10	0.0	1.2	3.0	5.2	3.0	6.4
14	2.2	2.0	3.1	1.3	5.3	3.3
17	5.3[b]	3.5[b]	2.6	1.5	7.9	5.1
18	2.5	2.0	3.2	4.5	5.7	6.5
19					4.7	4.8
20	1.6	1.7	3.4	2.9	5.0	4.6
24	1.9	1.7	2.3	3.1	4.2	4.8
25	1.6	1.5	4.0	4.1	5.6	5.6
26	1.7	0.9	1.3	2.0	3.1	2.8
27	1.8	1.6	3.1	2.7	4.9	4.3
30					3.5	3.3
33	2.5	1.6	2.1	2.2	5.0	4.5
34	2.2	1.5	0.6	0.3	2.8	1.8
35	1.6	2.5	2.0	2.1	3.6	4.6
Mean	1.60		2.55		4.36	
r	1.25		1.88		2.63	
SD_r	0.45		0.67		0.94	
RSD_r (%)	27.84		26.36		21.53	
R	1.71		3.11		3.74	
SD_R	0.61		1.11		1.34	
RSD_R (%)	38.28		43.50		30.70	

TABLE XXIX
Englyst Colorimetric Determination: Sample J + K = Cornflakes

Laboratory	Soluble Dietary fibre (i)	Soluble Dietary fibre (ii)	Insoluble Dietary fibre (i)	Insoluble Dietary fibre (ii)	Total Dietary fibre (i)	Total Dietary fibre (ii)
1	0.7	0.6	1.0	0.7	1.7	1.3
2	0.0	1.1	1.7	1.3	1.8	2.3
3	0.5	0.7	4.1	4.1	4.6	4.8
4	0.4	0.0	1.2	1.4	1.6	1.4
5	1.0	0.6	0.8	1.0	1.8	1.6
6	0.7	3.0	2.9	2.0	3.7	5.0
7	0.6	1.0	0.9	0.9	1.5	1.9
8	0.4	0.4	1.0	1.0	1.4	1.5
9	0.6	1.0	1.0	0.6	1.6	1.6
10	1.0	0.8	0.6	0.8	1.6	1.6
11	0.2	0.4	0.7	0.7	0.9	1.1
12	0.6	0.6	0.9	1.0	1.5	1.6
13	0.5	0.4	1.0	1.0	1.5	1.4
15	0.2	0.6	3.1	2.7	3.3	3.3
16	0.2	0.3	1.5	1.3	1.7	1.6
17	1.2	1.1	0.6	0.9	1.8	2.0
18	0.0	0.5	3.0	3.0	2.8	3.5
20	3.0[c]	3.4[c]	3.1	2.2	6.1	5.6
21	0.2	0.4	1.4	1.3	1.6	1.7
24	0.9	0.3		1.1	0.9	1.4
25	1.6	0.2	0.8	2.1	2.4	2.3
26	1.8	0.3	0.6	1.2	2.3	1.4
28	0.5	0.6	1.1	0.9	1.6	1.5
29	1.1	1.2	0.7	0.7	1.8	1.9
31	0.2	1.4	0.7	0.6	0.9	2.0
32	7.6[b]	0.0[b]	11.6[b]	1.4[b]	19.2[b]	1.4[b]
33	0.6	0.6	1.3	1.1	1.9	1.8
34	2.2	0.4	1.4	1.7	3.6	2.1
35	3.8[c]	3.9[c]	1.5	1.5	6.1	5.4
36	0.2		3.8	3.7	4.0	3.7
37	0.3	0.1	2.1	2.1	2.4	2.2

Mean	0.68	1.52	2.35
r	1.57	0.80	1.05
SD_r	0.56	0.28	0.37
RSD_r (%)	82.48	18.76	15.96
R	1.57	2.71	3.74
SD_R	0.55	0.97	1.34
RSD_R (%)	81.31	63.71	56.93

TABLE XXX
Englyst GLC Determination: Sample J + K = Cornflakes

Laboratory	Soluble Dietary fibre (i)	Soluble Dietary fibre (ii)	Insoluble Dietary fibre (i)	Insoluble Dietary fibre (ii)	Total Dietary fibre (i)	Total Dietary fibre (ii)
2	0.1[b]	1.2[b]	1.9	1.6	1.9	2.8
3	0.7	0.2	0.6[b]	2.2[b]	1.3	2.4
4	0.3	0.4	0.6	0.4	0.9	0.8
5	0.6	0.5	0.9	0.8	1.5	1.3
7	1.0	0.6	0.8	1.7	1.8	2.3
8	0.4	0.4	0.9	0.8	1.3	1.2
11	0.4	0.7	0.6	0.5	1.0	1.2
12	0.3	0.5	0.8	0.6	1.1	1.1
13	0.5	0.5	0.5	0.5	1.0	1.0
14	0.3	0.4	0.9	0.7	1.1	1.1
16	0.3	0.3	1.0	1.2	1.3	1.5
17	0.4	0.9	0.9	0.6	1.3	1.5
20	2.5[c]	2.9[c]	2.3	1.5	4.8[b]	4.4[b]
21	0.4	0.3	0.9	1.2	1.3	1.5
22	0.4	0.4	1.0	1.2	1.4	1.6
24	0.6	0.3	0.2	0.5	0.8	0.8
25	1.2	0.8	0.6	0.7	1.8	1.5
28	0.4	0.6	0.8	0.8	1.3	1.4
29	0.3	0.3	0.8	0.7	1.1	1.0
33	0.5	0.5	0.9	0.9	1.4	1.4
36	0.0	0.0	2.1	2.8	2.2	2.6
37	0.4	0.4	2.3	2.2	2.8	2.6
Mean	0.46		1.04		1.48	
r	0.47		0.69		0.73	
SD_r	0.17		0.25		0.26	
RSD_r (%)	36.38		23.78		17.56	
R	0.66		1.69		1.54	
SD_R	0.23		0.60		0.55	
RSD_R (%)	50.96		58.19		37.20	

TABLE XXXI
Prosky Determination: Sample J + K = Cornflakes

Laboratory	Soluble Dietary fibre (i)	Soluble Dietary fibre (ii)	Insoluble Dietary fibre (i)	Insoluble Dietary fibre (ii)	Total Dietary fibre (i)	Total Dietary fibre (ii)
7	0.0	0.0	2.6	2.2	2.6	2.2
9	0.2	0.2	3.1	2.8	3.3	3.0
10	0.0	0.0	3.0	3.2	3.0	3.2
14	0.2	0.2	3.3	3.2	3.5	3.4
17	1.6[c]	2.7[c]	2.4	2.9	4.1	5.6
18	0.7	1.0	3.5	4.1	4.2	5.1
19					4.1	4.3
20	0.0	0.0	3.5	3.8	3.5	3.8
24	0.1	0.3	2.8	1.6	2.9	1.9
25	0.1	0.0	5.9	5.2	6.0	5.2
26	0.1	1.1	1.9	1.3	1.9	2.4
27	0.0	0.0	3.3	3.2	3.3	3.2
30					2.4	2.1
33	0.2	0.6	2.8	2.5	4.0	3.2
34	0.6	0.0	2.6	1.7	3.2	1.7
35	2.3[b]	0.6[b]	3.4	3.3	5.7	3.9
Mean	0.23		3.04		3.50	
r	0.74		1.09		1.69	
SD_r	0.26		0.39		0.60	
RSD_r (%)	112.71		12.76		17.23	
R	0.92		2.77		3.21	
SD_R	0.33		0.99		1.15	
RSD_R (%)	140.49		32.53		32.81	

TABLE XXXII
Englyst Colorimetric Determination: Sample L + M = Wholemeal Bread

Laboratory	Soluble Dietary fibre (i)	Soluble Dietary fibre (ii)	Insoluble Dietary fibre (i)	Insoluble Dietary fibre (ii)	Total Dietary fibre (i)	Total Dietary fibre (ii)
1	2.6	2.7	7.0	6.9	9.7	9.6
2	2.4	2.6	7.2	7.0	9.6	9.7
3	2.0	2.5	8.8	8.8	10.8	11.3
4	4.9[c]	5.2[c]	6.1	4.4	11.0	10.1
5	4.1	1.9	8.4	9.4	12.5	11.3
7	2.5	2.7	5.7	5.7	8.2	8.4
8	2.1	2.2	8.1	8.1	10.2	10.3
9	2.6	3.3	7.4	7.8	10.0	11.1
10	3.2	4.0	7.7	7.7	10.9	11.7
11	2.9	2.7	6.3	6.2	9.2	8.9
12	3.4	3.5	8.2	7.9	11.6	11.4
13	2.6	2.6	7.8	8.2	10.4	10.8
14	3.4	3.0	6.8	6.8	10.2	9.8
15	3.1	3.5	8.6	8.3	11.8	11.7
16	3.4	3.2	8.0	8.2	11.4	11.4
17	2.2	1.9	8.7	7.4	10.8	9.3
18	2.8	2.8	7.8	7.5	10.6	10.3
20	3.3	2.6	8.6	10.8	11.9	13.4
21	2.9	2.6	8.6	8.6	11.2	11.5
24	2.2	3.4	6.1	6.0	8.3	9.4
25	1.8	2.9	7.9	6.9	9.7	9.8
26	3.6[b]	0.5[b]	5.6	8.2	9.2	8.7
28	3.0	2.9	8.1	8.4	11.1	11.4
29	2.3	3.1	7.8	7.3	10.1	10.4
31	3.2	2.6	8.8	8.3	12.0	10.9
32	2.2	3.7	6.6	6.6	8.8	10.3
33	3.0	3.2	7.8	8.0	10.8	11.2
34	2.9	3.1	8.0	8.1	10.9	11.2
35	3.6	4.9	6.8	7.7	10.4	12.6
36		17.6	11.9	10.0	11.9[b]	27.6[b]
37	2.8	2.6	8.0	7.7	10.8	10.2
Mean	2.88		7.71		10.54	
r	1.45		1.74		1.62	
SD_r	0.52		0.62		0.58	
RSD_r (%)	18.05		8.07		5.50	
R	1.64		3.43		3.08	
SD_R	0.59		1.22		1.10	
RSD_R (%)	20.36		15.88		10.43	

TABLE XXXIII
Englyst GLC Determination: Sample L + M = Wholemeal Bread

Laboratory	Soluble Dietary fibre (i)	Soluble Dietary fibre (ii)	Insoluble Dietary fibre (i)	Insoluble Dietary fibre (ii)	Total Dietary fibre (i)	Total Dietary fibre (ii)
2	1.7[c]	0.7[c]	7.5	8.4	9.3	9.2
3	2.1	2.2	7.2	6.7	9.3	8.9
4	3.2	1.9	5.9	6.5	9.1	8.4
5	3.2	2.3	7.3	7.7	10.5	10.0
7	1.7	2.9	8.0	7.4	9.7	10.3
8	2.1	2.1	7.3	7.3	9.4	9.4
11	2.4	2.9	5.8	5.6	8.2	8.5
12	2.8	2.4	7.2	7.0	10.0	9.4
13	2.7	2.4	6.7	7.2	9.4	9.6
14	3.0	2.8	6.5	6.4	9.5	9.2
16	3.1	2.7	6.7	7.0	9.8	9.7
17	2.5	2.2	6.8	6.8	9.3	9.0
20	2.7	2.8	6.7	8.3	9.4	11.1
21	1.7[c]	1.8[c]	7.9	7.9	9.6	9.7
22	2.7	2.0	6.9	7.0	9.6	9.1
24	2.2	3.0	5.6	5.8	7.8	8.8
25	2.2	2.5	5.9	5.6	8.1	8.1
28	2.5	2.8	7.4	7.4	9.9	10.2
29	1.6	2.5	8.0	7.7	9.6	10.2
33	2.5	2.6	6.8	7.0	9.2	9.6
36	3.1[b]	17.3[b]	8.3	7.3	11.4[b]	20.4[b]
37	2.3	2.6	7.1	7.1	9.4	9.7
Mean	2.50		7.01		9.39	
r	1.20		1.05		1.13	
SD_r	0.43		0.38		0.40	
RSD_r (%)	17.11		5.35		4.29	
R	1.20		2.10		1.88	
SD_R	0.43		0.75		7.15	
RSD_R (%)	17.11		10.68		0.67	

TABLE XXXIV
Prosky Determination: Sample L + M = Wholemeal Bread

Laboratory	Soluble Dietary fibre (i)	Soluble Dietary fibre (ii)	Insoluble Dietary fibre (i)	Insoluble Dietary fibre (ii)	Total Dietary fibre (i)	Total Dietary fibre (ii)
7	2.4	2.7	8.7	8.6	11.1	11.3
9	1.9	2.7	8.7	9.2	10.7	11.9
10	1.6	0.0	11.5	9.8	13.1	9.8
14	1.8	0.3	9.6	10.6	11.4	10.9
17	1.4	2.9	8.9	9.8	10.3	12.7
18	2.1	2.3	10.2	9.3	12.3	11.6
19					12.2	11.9
20	2.1	2.0	9.9	11.4	12.0	13.4
24	1.8	2.4	10.1	12.7	11.9	15.1
25	1.9	1.5	13.1	13.8	15.0	15.3
26	1.0	0.0	6.4	8.9	7.4	8.9
27	2.1	2.2	10.4	10.8	12.5	13.0
30					10.8	10.3
33	2.1	1.7	9.3	9.9	13.4	13.0
34	2.6	1.9	8.3	8.1	10.9	10.0
35	2.0	1.8	10.8	11.1	12.8	12.9
Mean	1.83		10.00		11.87	
r	1.68		2.49		2.93	
SD_r	0.60		0.89		1.05	
RSD_r (%)	32.78		8.90		8.81	
R	2.08		4.49		4.85	
SD_R	0.74		1.60		1.73	
RSD_R (%)	40.64		16.05		14.60	

TABLE XXXV
Englyst Colorimetric Determination:
Sample N + O = Mix White Bread + Wholemeal Bread

Laboratory	Soluble Dietary fibre (i)	Soluble Dietary fibre (ii)	Insoluble Dietary fibre (i)	Insoluble Dietary fibre (ii)	Total Dietary fibre (i)	Total Dietary fibre (ii)
1	3.4	2.6	4.6	4.6	8.0	7.3
2	2.4	2.6	4.2	4.3	6.6	6.8
3	2.0	2.0	6.7	6.4	8.7	8.4
4	2.9	3.2	4.2	4.2	7.1	7.4
5	3.6	2.8	5.4	5.8	7.2	8.6
7	2.5	2.0	3.2	3.2	5.7	5.2
8	2.0	1.7	5.4	4.8	7.4	6.5
9	2.4	3.7	5.1	4.0	7.5	7.7
10	2.8	2.7	4.6	4.7	7.4	7.4
11	2.3	2.3	3.9	4.0	6.2	6.3
12	1.6	2.9	6.0	4.7	7.6	7.6
13	2.6	3.1	4.8	4.3	7.4	7.4
14	3.0	2.4	4.5	4.8	7.5	7.2
15	3.0	2.9	5.6	5.8	8.6	8.6
16	2.7	2.4	5.2	5.0	7.9	7.4
17	2.6	1.7	4.9	5.4	7.5	7.1
18	3.2	2.2	4.1	5.0	7.3	7.2
20	3.3	3.0	5.4	6.1	8.7	9.1
21	2.5	2.4	5.3	5.3	7.8	7.7
24	2.6	2.7	2.9	2.8	5.5	5.5
25	2.3	1.6	4.3	5.5	6.6	7.1
26	2.4	2.3	3.9	3.8	6.2	6.1
28	2.5	2.5	5.0	5.0	7.6	7.5
29	2.5	3.3	4.5	4.4	7.0	7.7
31	3.4	2.6	5.3	5.6	8.7	8.0
32	2.1	2.7	4.7	4.2	6.8	6.8
33	2.8	2.4	5.2	5.5	8.0	8.0
34	2.6	2.7	5.2	5.3	7.8	8.0
35	3.0	3.8	5.0	5.0	8.0	8.8
36	13.1[(b)]	3.9[(b)]	6.9	6.9	19.9[(b)]	10.8[(b)]
37	3.4	3.3	4.6	4.7	8.4	7.9
Mean	2.65		4.87		7.42	
r	1.18		0.97		0.93	
SD_r	0.42		0.35		0.33	
RSD_r (%)	15.97		7.11		4.49	
R	1.42		2.43		2.44	
SD_R	0.51		0.87		0.87	
RSD_R (%)	19.16		17.82		11.74	

TABLE XXXVI
Englyst GLC Determination:
Sample N + O = Mix White Bread + Wholemeal Bread

Laboratory	Soluble Dietary fibre (i)	Soluble Dietary fibre (ii)	Insoluble Dietary fibre (i)	Insoluble Dietary fibre (ii)	Total Dietary fibre (i)	Total Dietary fibre (ii)
2	1.3	2.0	5.2	5.1	6.4	7.1
3	1.9	1.9	4.4	4.3	6.3	6.2
4	1.2	1.5	5.0	4.1	6.0	5.6
5	2.5	2.1	5.0	5.1	7.5	7.2
7	2.5	2.5	4.7	4.1	7.2	6.6
8	1.9	1.9	4.6	4.1	6.5	6.0
11	2.7	1.9	3.7	3.7	6.4	5.6
12	2.0	1.8	4.6	4.3	6.6	6.1
13	2.2	2.4	4.3	4.1	6.5	6.5
14	2.6	2.2	4.4	4.6	7.0	6.8
16	2.4	2.2	4.4	4.2	6.8	6.3
17	1.9	2.0	4.7	4.4	6.6	6.4
20	3.5	2.2	4.5	5.5	8.1	7.7
21	2.3	2.6	4.9	4.4	7.2	7.0
22	2.5	2.3	4.4	4.3	6.8	6.5
24	2.2	2.2	3.4	3.5	5.6	5.7
25	2.2	2.2	3.5	3.5	5.7	5.7
28	2.4	1.8	4.3	4.4	6.6	6.3
29	2.2	0.8	4.8	5.0	7.0	5.8
33	2.3	2.1	4.5	4.5	6.8	6.6
36	1.2	3.0	14.2[b]	5.3[b]	15.3[b]	8.2[b]
37	2.3	3.2	4.6	4.1	7.0	7.2
Mean	2.16		4.41		6.56	
r	1.33		0.79		0.92	
SD_r	0.47		0.28		0.33	
RSD_r (%)	21.97		6.38		4.98	
R	1.39		1.37		1.67	
SD_R	0.50		0.49		0.60	
RSD_R (%)	23.03		11.09		9.09	

TABLE XXXVII
Prosky Determination:
Sample N + O = Mix White Bread + Wholemeal Bread

Laboratory	Soluble Dietary fibre (i)	Soluble Dietary fibre (ii)	Insoluble Dietary fibre (i)	Insoluble Dietary fibre (ii)	Total Dietary fibre (i)	Total Dietary fibre (ii)
7	0.9	1.5	3.0[(b)]	6.2[(b)]	3.9[(b)]	7.7[(b)]
9	1.1	1.8	6.0	5.4	7.0	7.3
10	1.5	1.5	7.5	7.5	9.0	9.0
14	2.1	1.0	6.5	6.8	8.6	7.8
17	3.5	4.4	6.2	5.3	9.7	9.7
18	2.1	2.1	7.0	7.4	9.1	9.5
19					8.9	9.1
20	2.0	2.3	6.7	6.9	8.6	9.2
24	2.1	0.9	8.1	7.9	10.2	8.7
25	1.9	2.0	8.0	8.7	9.9	10.7
26	0.0	0.0	4.7	4.9	4.7	4.9
27	2.1	2.0	6.6	7.6	8.7	9.6
30					6.8	6.5
33	1.4	1.7	6.5	6.2	8.8	9.0
34	3.9	1.9	4.7	4.3	8.6	6.2
35	3.4	2.7	6.1	6.4	8.5	9.1
Mean	1.93		6.53		8.45	
r	1.59		1.01		1.72	
SD_r	0.57		0.36		0.61	
RSD_r (%)	29.47		5.51		7.26	
R	2.87		3.29		4.12	
SD_R	1.02		1.18		1.47	
RSD_R (%)	52.18		17.99		17.44	

TABLE XXXVIII

Key to Tables II to XXXVII

All results expressed as g / 100 g

a:	Single result reported, not used in calculation of mean, repeatability or reproducibility.
b:	Outlying result by Cochran's test at P<0.01 level, not used in calculation of mean, repeatability or reproducibility.
c:	Outlying result by Grubb's Test at P<0.01 level, not used in calculation of mean, repeatability or reproducibility.
r:	Repeatability (within laboratory variation). The value below which the absolute difference between two single test results obtained with the same method on identical test material under the same conditions may be expected to lie with 95% probability.
SD_r	The standard deviation of the repeatability.
RSD_r %:	The relative standard deviation of the repeatability. $SD_r \times 100 / x$
R:	Reproducibility (between laboratory variation). The value below which the absolute difference between two single test results obtained with the same method on the identical test material under different conditions may be expected to lie with 95% probability.
SD_R	The standard deviation of the reproducibility.
RSD_R %:	The relative standard deviation of the reproducibility $SD_R \times 100 / x$

TABLE XXXIX
Summary of Results for Individual Samples

Sample	Code	Method	Fibre Type	Mean	r	R
Coconut	A	E-GLC	Soluble	1.21	1.46	2.54
			Insoluble	11.12	1.61	3.10
			Total	12.31	2.61	3.03
		E-COL	Soluble	1.39	1.09	2.82
			Insoluble	11.71	0.95	4.62
			Total	13.06	0.91	4.83
		PROSKY	Soluble	0.31	0.41	0.71
			Insoluble	13.38	1.86	5.64
			Total	16.41	3.37	17.00
Porridge	B	E-GLC	Soluble	3.68	1.35	1.94
			Insoluble	3.42	0.77	1.46
			Total	7.22	1.13	2.27
		E-COL	Soluble	4.46	1.56	2.38
			Insoluble	4.07	0.65	2.67
			Total	8.69	0.72	2.21
		PROSKY	Soluble	1.79	0.64	3.99
			Insoluble	5.33	1.30	1.84
			Total	7.27	1.07	4.93
Baked Beans	C	E-GLC	Soluble	7.06	1.27	1.95
			Insoluble	5.56	0.86	1.80
			Total	12.62	1.07	3.06
		E-COL	Soluble	7.29	1.88	3.00
			Insoluble	6.16	0.89	2.65
			Total	13.13	4.52	4.52
		PROSKY	Soluble	5.96	0.66	2.57
			Insoluble	11.00	0.83	1.79
			Total	16.10	2.87	5.34
Cabbage	D	E-GLC	Soluble	12.15	4.24	6.61
			Insoluble	13.04	1.80	4.93
			Total	25.25	3.17	8.54
		E-COL	Soluble	9.93	3.07	5.05
			Insoluble	13.61	1.16	4.59
			Total	23.80	2.89	5.92
		PROSKY	Soluble	6.05	2.14	4.86
			Insoluble	21.98	3.13	6.66
			Total	27.12	2.66	6.47

TABLE XXXIX
Summary of Results for Individual Samples

Sample	Code	Method	Fibre Type	Mean	r	R
Mashed	E	E-GLC	Soluble	4.03	0.84	0.97
			Insoluble	2.83	0.84	1.29
			Total	6.87	0.67	1.44
		E-COL	Soluble	3.85	0.75	0.97
			Insoluble	2.94	0.79	1.80
			Total	7.12	0.89	2.81
		PROSKY	Soluble	2.19	0.90	1.67
			Insoluble	5.62	1.33	1.77
			Total	7.67	1.40	2.17
Banana	F	E-GLC	Soluble	2.88	0.80	1.57
			Insoluble	1.87	0.65	0.97
			Total	4.62	0.76	1.48
		E-COL	Soluble	2.73	1.60	1.66
			Insoluble	2.46	0.93	1.79
			Total	5.40	1.02	2.61
		PROSKY	Soluble	1.94	1.00	2.28
			Insoluble	5.35	1.44	2.53
			Total	7.95	2.01	6.79
Apple	G	E-GLC	Soluble	5.28	1.21	2.21
			Insoluble	6.45	1.10	2.45
			Total	11.73	1.06	3.79
		E-COL	Soluble	4.13	2.16	3.50
			Insoluble	6.93	0.89	3.83
			Total	10.78	1.55	6.23
		PROSKY	Soluble	3.78	0.99	2.07
			Insoluble	8.42	0.73	1.21
			Total	12.36	1.75	2.33
Bread	H	E-GLC	Soluble	2.82	0.61	1.61
			Insoluble	2.77	0.48	0.96
			Total	5.61	0.66	1.96
		E-COL	Soluble	3.39	1.28	1.43
			Insoluble	3.18	0.97	1.23
			Total	6.57	0.83	1.98
		PROSKY	Soluble	2.42	1.35	2.30
			Insoluble	4.29	0.90	2.26
			Total	6.83	1.25	3.15

TABLE XXXIX
Summary of Results for Individual Samples

Sample	Code	Method	Fibre Type	Mean	r	R
White Bread	I/P	E-GLC	Soluble	1.83	1.50	1.50
			Insoluble	1.61	0.74	0.91
			Total	3.43	0.99	1.33
		E-COL	Soluble	2.11	1.78	1.92
			Insoluble	2.00	1.05	1.35
			Total	4.16	1.62	2.42
		PROSKY	Soluble	1.60	1.25	1.71
			Insoluble	2.55	1.88	3.11
			Total	4.36	2.63	3.74
Cornflakes	J/K	E-GLC	Soluble	0.46	0.47	0.66
			Insoluble	1.04	0.69	1.69
			Total	1.48	0.73	1.54
		E-COL	Soluble	0.68	1.57	1.57
			Insoluble	1.52	0.80	2.71
			Total	2.35	1.05	3.74
		PROSKY	Soluble	0.23	0.74	0.92
			Insoluble	3.04	1.09	2.77
			Total	3.50	1.69	3.21
Wholemeal	L/M	E-GLC	Soluble	2.50	1.20	1.20
			Insoluble	7.01	1.05	2.10
			Total	9.39	1.13	1.88
		E-COL	Soluble	2.88	1.45	1.64
			Insoluble	7.71	1.74	3.43
			Total	10.54	1.62	3.08
		PROSKY	Soluble	1.83	1.68	2.08
			Insoluble	10.00	2.49	4.49
			Total	11.87	2.93	4.85
1:1 Bread	N/O	E-GLC	Soluble	2.16	1.33	1.39
			Insoluble	4.41	0.79	1.37
			Total	6.56	0.92	1.67
		E-COL	Soluble	2.65	1.18	1.42
			Insoluble	4.87	0.97	2.42
			Total	7.42	0.93	2.44
		PROSKY	Soluble	1.93	1.59	2.87
			Insoluble	6.53	1.01	3.29
			Total	8.45	1.72	4.12

TABLE XXXX
Overall Summary of Results by Method

Method		Number of results (labs)	Mean No. of Results per sample	Dietary Fibre	Reproducibility R95 (mean)
Englyst	(GLC)	420	22	8.57	2.56
	Total	418	22	3.66	1.96
	Soluble	428	22	4.99	1.89
	Insoluble				
Englyst	(Colorimetry)				
	Total	580	31	8.99	3.47
	Soluble	576	31	3.70	2.23
	Insoluble	574	31	5.36	2.70
Prosky	(Gravimetric)				
	Total	278	16	10.20	5.1
	Soluble	242	14	2.38	2.24
	Insoluble	258	14	7.65	3.15