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

Part I: Initial Trial

JOHN H. CUMMINGS†, HANS N. ENGLYST† AND ROGER WOOD‡§

† MRC Dunn Clinical Nutrition Centre, Old Addenbrooke's Hospital, Trumpington Street, Cambridge CB2 1QE and ‡Ministry of Agriculture, Fisheries and Food, 65 Romney Street, London SW1P 3RD.

A collaborative trial in 22 laboratories to assess potential legislative methods for the determination of dietary fibre in cereals and cereal products has been carried out. Five methods were tested including: the Association of American Cereal Chemists (AACC) (first approval) method for insoluble dietary fibre in cereals (Neutral Detergent Fibre); a simple method for "dietary fibre" based on that of McCance, Widdowson and Shackleton (1936) (MWS); the Englyst procedure; ISO procedure for the determination of bran by an enzymic method; and the Association of Official Analytical Chemists (AOAC) procedure for the determination of dietary fibre. Crude fibre was included in the trial for reference purposes. Seven samples including flours, biscuits, breakfast cereals, soya and bran were prepared for the trial and analysed by the laboratories. Widely differing results were obtained by each method and amongst the varying collaborators with any one method so that no one technique was found to be satisfactory as a potential statutory method. The results do suggest, however, that the Englyst procedure is the most accurate and gives valuable information on the composition of dietary fibre in addition to total values. It is suggested that this method be simplified and validated in a further collaborative study.

Although the importance of fibre in animal nutrition has been recognised for many years, it is only in recent times that its significance for man has become apparent. In 1971 Burkitt[1], a surgeon who at that time had recently returned from a lifetime of work in Africa, wrote a paper describing in detail the geographical variation in mortality from bowel cancer across the world, and pointed out that these contrasts could largely be accounted for by differences in

* Collaborators (Initial trial): E. Anderson, H. Bee, M. Billington, R. Cooke, P. Cox, A. Croft, D. Crudgington, R. Fawcett, N. Fisher, J. Hamence, A. Harrison, A. Hudson, A. Law, P. Martin, R. Mongeau, A. Phillips, L. Prosky, J. Shelton, D. Southgate, R. Stevens and D. Symonds.
§ To whom correspondence should be addressed.

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dietary fibre intakes of the various populations, a low intake predisposing to the development of cancer. In the following year Trowell[2], also recently returned from Africa, postulated on the basis of similar evidence that fibre could protect also against the development of ischaemic heart disease, the principal cause of death in Western industrialised society. Subsequently Burkitt and Trowell published a book[3] in which evidence was presented that dietary fibre deficiency was the cause of a wide range of human diseases.

In the past 10 to 15 years a great deal of research has been done to test the dietary fibre hypothesis. Some further epidemiological studies lend support to the view that fibre, and especially the pentose fraction of it, may be protective against bowel cancer[4,5] and that cereal dietary fibre, which is rich in pentose, may protect against ischaemic heart disease[6]. Physiological studies have shown that fibre is an important dietary component with a range of effects on gastrointestinal function and metabolism[7,8]. Certain forms of fibre, particularly the gel-forming polysaccharides such as pectin and guar gum, alter glucose absorption and insulin metabolism[9,10], whilst others modify bile acid and steroid metabolism[11,12]. Fibre is extensively degraded in the human gut, the rate and extent of its degradation depending on such factors as its water solubility and lignification[13]. It is a primary determinant of large bowel function but its effect varies considerably with source of fibre and its chemical composition[14]. The uronic acid component of fibre may also bind divalent cations in the gut[15].

Definition of Fibre

It was apparent very early in the development of the dietary fibre hypothesis that fibre could not be defined for human nutrition as crude fibre[16] since crude fibre bears little relation to the plant cell wall and other polysaccharides that are associated with physiological changes in man. In 1972 Trowell redefined fibre as "the skeletal remains of plant cells that are resistant to digestion by enzymes of man"[17], and further modified the definition in 1976[18] to "the plant polysaccharides and lignin which are resistant to hydrolysis by the digestive enzymes of man".

With the development of interest in dietary fibre has come the need for an accurate analytical method. A number of attempts to produce such a method have been made, which have as their aim the determination of dietary polysaccharides and other plant material escaping breakdown by the digestive enzymes of man, using a combination of enzymic hydrolysis and gravimetric analysis[19–22]. Such an approach is full of problems, however, because it is not known which polysaccharides resist digestion by human enzymes. Moreover, the intestinal milieu varies from person to person, making it difficult to reproduce in the laboratory. In any case, a physiological definition is an inappropriate objective for an analytical method since the two disciplines see fibre from different viewpoints. It was therefore decided to define dietary fibre for analytical purposes in terms of its chemical components[23], the principal of which are polysaccharides.

The human diet contains two main classes of polysaccharides, starch and dietary fibre polysaccharides which may conveniently be called non-starch polysaccharide (NSP) (i.e. non α-glucan polysaccharides). The only plant

polysaccharide known to be hydrolysed by the digestive enzymes of man is starch. Trowell's 1976 definition of fibre is therefore equivalent to defining it as plant polysaccharides other than starch, or as non-starch polysaccharides and lignin. It is NSP which has become the principal objective of recent analytical methods[24,25]. These methods give detailed information about the broad classes of polysaccharide contained in any source of NSP and also its monosaccharide composition. Such information is valuable in interpreting physiological studies in man, which have already shown that the properties of fibre are probably determined by its chemical structure. It also allows the origin of fibre in plant foods to be ascertained and the addition of purified cell wall material, modified starch, cellulose and other polysaccharides to be identified.

Lignin has not been included in the analytical definition of dietary fibre. Physiologically, lignin may be an important cell wall component since it has been shown to inhibit fermentation in animal studies[26]. However it is a minor contributor to the human diet; with the exception of whole-wheat cereals few foods contain measurable quantities. Furthermore it is difficult to analyse chemically, as the results from this trial also indicate, and none of the present dietary fibre methods which include a value for lignin can in fact justify this on strict chemical grounds. All these methods are isolating a collection of inert material which is better referred to as "substances measuring as lignin"; the inclusion of an imprecise lignin value alongside accurate measurement of NSP is considered to be self-defeating. If lignin is shown to be an important dietary constituent in the future then it should be measured separately, as should be any other plant cell wall associated substance such as silica, cutin, etc.

Resistant Starch

One of the principal problems in the measurement of NSP is the removal of starch. Starch which is not removed after gelatinisation and enzymic hydrolysis will be measured as NSP, partly as cellulose and partly as non-cellulosic glucose[24]. Starch which resists hydrolysis after gelatinisation in water at 100°C is commonly called resistant starch (RS). Significant amounts of RS are found in processed foods such as cornflakes[27]. Because of its resistance to enzymic digestion *in vitro* it has been suggested that RS may act like fibre in the gut and therefore should legitimately be included as part of dietary fibre. From the analytical viewpoint, however, this is undesirable since chemically it is starch, not NSP, and can be measured as such[28]. Furthermore, the amounts of RS in food vary depending on the treatment given to the food before and during analysis, including such procedures as cooling, freezing, drying and solvent extraction[24]. Its inclusion, therefore, in any analytical method used for legislative purposes would lead to confusion and disputes. Starch may resist hydrolysis with α-amylase in the small intestine and in *in vitro* incubations for a variety of reasons. Starch granules from potato, banana, high amylose corn, legumes and also the small granules in wheat show considerable resistance to hydrolysis with α-amylase *in vitro*. Nevertheless, neither these nor starch retrograded during food processing are strictly resistant. They can all eventually be degraded by α-amylase after suitable treatment showing that they are truly starch and not non-starch polysaccharides. Starch may also escape digestion in

the small intestine because of the presence of α-amylase inhibitors from foods or due to complexes formed with other food components such as fat, protein or simply due to excess intake of starch in a less available form as in unmilled seeds and grains. It is of considerable interest for metabolism to know the amount of starch which, for these reasons, may resist or escape digestion in the small intestine. However this is a separate problem from the measurement of dietary fibre.

Legislation

An accurate method of measuring dietary fibre is needed not only for the interpretation of physiological and epidemiological studies in man, but also for legislative purposes such as food labelling. In 1981 the Department of Health and Social Services' Committee on Medical Aspects of Food Policy panel on the Nutritional Value of Bread and Flour recommended that information relating to the "cereal dietary fibre content of bread should be made available to the consumer by an agreed form of labelling"[29]. The public's desire for nutritional labelling of all food, not just cereals, is increasing and with the considerable interest in dietary fibre it is likely that the demand for information on the fibre content of a wide range of foodstuffs will increase.

For statutory purposes, however, there must be an agreed definition of fibre. In the light of what has been said it is proposed that dietary fibre be defined as the sum of non-starch polysaccharides in food; that is, polysaccharides which after dispersion resist hydrolysis by mammalian α-amylase (E.C. 3.2.1.1). Such a definition may be expected to give analytical values closely related to the current physiological concept of dietary fibre and can be achieved by presently available techniques[28]. Lignin and RS are excluded for the reasons already given.

The Ministry of Agriculture, Fisheries and Food (MAFF), in view of the above, decided to investigate the possibility of deciding upon a method for dietary fibre which could be discussed with a view to including it in legislation, or Code of Practice, appropriate to Bread and Flour.

To this end a small analytical Advisory Panel was set up under Ministry auspices and to which representatives from most of the main interested and informed centres dealing with dietary fibre analysis were invited.

It was decided by the Panel to survey the available methods and to organise a collaborative trial to assess some of them. This was considered to be necessary not only to obtain the analytical performance characteristics of the various methods but also to enable enforcement analysts, in particular, to become familiar with the alternative procedures considered. Collaborative trials to test or verify methods of analysis for foodstuffs of significance to UK analysts have been organised by MAFF. The results of such trials are normally published as Reports; the aims and objectives of the trials are given in a previous Report[16].

It was anticipated by the Advisory Panel that it might be necessary to carry out a sequence of collaborative trials to assess potential methods of analysis for dietary fibre. The initial results which were obtained are being given in this Part I of the Report on the collaborative trial work that has been carried out.

Organisation of the Initial Trial

LABORATORIES

A total of 22 analysts participated in the trial. As there were a number of methods being tested, analysts were permitted to choose whichever methods they wished to use but all were to express their results on a dry-matter basis. As a result each method was tested by a different number of analysts and this is reflected in the results tables.

METHODS

There were five "dietary fibre type" methods, 1 crude fibre method, 1 lignin method and a moisture method tested in the trial. The methods tested were:

(a)–(e) Dietary fibre

(a) The Association of American Cereal Chemists (AACC) (First Approval) method for insoluble dietary fibre in cereals (Neutral Detergent Fibre) (NDF)[30].
(b) A simple procedure based on a modification of that of McCance, Widdowson and Shackleton (MWS). The method is given in detail in Appendix I.
(c) Dietary fibre measured as non-starch polysaccharides, developed in the Dunn Clinical Nutrition Laboratory and referred to as the "Englyst" procedure[24]. The method is given in detail in Appendix II.
(d) ISO procedure for the determination of bran by an enzymic method[31].
(e) "Association of Official Analytical Chemists (AOAC)" procedure for the determination of dietary fibre[22].

(f) Crude fibre

A crude fibre method was included in the trial for reference purposes. The method chosen was that based on the work of Van de Kamer and Van Ginkel[32].

It has already been subjected to a collaborative trial organised by MAFF as it was a strong candidate for a legislative method when it seemed likely that the European Community (EC) would prescribe such a criterion in the then proposed Bread Directive. The results from that trial are available[16].

(g) Moisture

The moisture determination was carried out by drying to a constant weight at 103 ± 2°C in an atmospheric pressure drying oven.

(h) Lignin

The Morrison method[33] for lignin was used. In this method the sample is dried and prepared by dissolving in acetyl bromide. The absorption was determined spectrophotometrically at 280 nm and the lignin content calculated from a given generalised regression equation.

The dietary fibre methods (a–e) are commented on in detail in the discussion section of this Report.

Other dietary fibre methods, for example those of Southgate[34,35], which have been used to obtain the values of dietary fibre given in the National Food Tables[36], and Selvendran and Du Pont[37,38] were considered for inclusion in the trial but were rejected as either being too complex in themselves or of making the trial too complex. Nevertheless details of these two methods and specific comments on them are given in Appendix III.

SAMPLES

Seven samples were prepared for the trial the results of which are now being reported. They were as follows: (A) white flour; (B) brown flour; (C) wholemeal digestive biscuits; (D) cornflakes; (E) bran; (F) instant porridge cereal; (G) soya flour. The samples were thoroughly ground and homogenised before dispatch to the participants.

Results

Each analyst received the seven trial samples and was asked to carry out their analyses in duplicate, i.e. as "known" duplicates.

The results which were obtained by the participating analysts are given in Tables I to VII below.

STATISTICAL ANALYSIS OF THE RESULTS

The results obtained were statistically analysed for outlying results by the Cochran's and Dixon's Tests using the procedures given by the British Standards Institution[39]. Some outlying results were identified and these were not used in the calculation of the means ($\bar{x}$), repeatabilities (r) or reproducibilities (R).

The final values of the means, repeatabilities and reproducibilities are given in Tables I to VII and have been collated for convenience in Table IX. The values are as defined and calculated by the procedures described by the British Standards Institution[39].

In addition, Table IX shows the standard deviation of the single Englyst method values which were obtained.

Discussion

Discussion and comment is made on each of the "dietary fibre" methods tested:

THE ASSOCIATION OF AMERICAN CEREAL CHEMISTS (FIRST APPROVAL) METHOD FOR INSOLUBLE DIETARY FIBRE IN CEREALS (NEUTRAL DETERGENT FIBRE) (NDF)[30]

This is based on the well-established and widely used method developed by Van Soest[40], and was originally intended as a replacement for the crude fibre method for animal feeds. More recently, however, it has been used for human food. In the method free sugars, starch, soluble cell wall polysaccharides, fat and protein are removed with hot neutral detergent, and fibre is measured as the weight of the remainder (less ash). The method has been modified by Schaller[41] and Robertson and Van Soest[42] to include a pre-incubation with α-amylase in order to remove starch more adequately from starch-rich food. When the NDF

TABLE I

DIETARY FIBRE COLLABORATIVE TRIAL RESULTS

WHITE FLOUR SAMPLE

Laboratory	Dietary fibre *g/100 g* (d. wt basis): AACC method		MWS method		Englyst method	ISO method		AOAC method		Crude fibre *g/100 g* (d. wt basis)		Moisture *g/100 g*		Lignin *g/100 g*
1	1·34	1·46	12·61[c]	—	3·19	1·00	0·77	3·24[c]	—	0·27	0·27	11·18	11·18	—
2	1·20	1·13	5·51	4·27	3·06							10·03	10·09	(0·57)
3	3·42[b]	3·36[b]	6·00	5·78		0·87	0·93	7·87[a]	2·74[a]	1·22[b]	1·16[b]	11·38	11·40	
4					3·29									
5	1·68[a]	4·56[a]								0·20	0·25	10·74	10·81	
6								2·97[c]						
7	1·35	1·70	12·42	13·22				5·57	5·37			10·54	10·71	
8	1·03	1·30	4·57	4·53	3·88			3·66	3·17			10·21	10·18	(0·00, 0·00)
9	—	—	14·71	11·87			—	4·41[c]	—			11·33	11·30	
10	1·58	1·29	75·68[b]	75·66[b]		34·01[c]	—					9·63	9·39	
11	0·46	0·38	37·90	34·52		0·19	0·41	4·01	4·13	0·21	0·21	9·61	9·58	
12	0·11	0·11	0	2·86				3·21	2·83			10·92	10·86	
13	0·83	0·17	29·50	27·53		0·06	0·06	3·03	3·61			10·63	10·46	
14	7·56[b]	7·13[b]										9·76[a]	10·43[a]	
15	1·40	1·40	7·76	7·73		2·14	2·40	2·94	3·08	0·56	0·46	10·70	10·85	
16	1·34	1·65	8·83	8·42				2·97	2·86			10·66	10·89	
17	0·87	0·74	4·34	8·01		1·78[a]	0·34[a]	0[b]	0·97[b]	0·19	0·20	10·80	10·88	(0·00, 0·00)
18	1·22	0·88	13·64	12·22		0·93	0·78	4·90	5·24			10·39	10·37	
19	1·44	1·49	24·78	29·55	3·02	0·16	0·04	3·02[c]	—	0·19	0·21	10·46	10·48	(0·82, 1·91)
20	1·55	1·80	9·55	8·99	2·88							11·00	11·16	(−0·5, −0·71)
21	1·54	1·49						3·52[a]	13·24[a]			11·01[c]	—	
22	1·49	1·39	7·76	9·72	4·94	1·26	1·08	4·19	4·09	0·46	0·47	10·92	10·95	(0·55, 0·56)
Mean $\bar{x}$	1·16		12·55		3·47	0·82		3·83		0·30		10·63		—
Repeatability r	0·51		4·51		—	0·35		0·64		0·09		0·23		—
Reproducibility R	1·36		28·70		—	2·04		2·68		0·38		1·50		—

For key, see Table VIII.

TABLE II

DIETARY FIBRE COLLABORATIVE TRIAL RESULTS

ADDED—BRAN FLOUR SAMPLE

Laboratory	Dietary fibre *g/100 g* (d. wt basis) AACC method 1		MWS method		Englyst method	ISO method		AOAC method		Crude fibre *g/100 g* (d. wt basis)		Moisture *g/100 g*		Lignin *g/100 g*
1	9·30	9·40	16·54[c]		7·88	9·87	9·67	11·50	—	1·75	1·80	12·45	12·43	(0·62)
2	9·52	9·59	15·39	13·26	7·85							11·78	12·07	(1·07)
3	10·33	10·21	7·90	7·28		8·48	7·47	12·29	11·16	2·80	2·80	12·41	12·43	
4					9·72									(—)
5	9·45[a]	13·64[a]								1·69[a]	1·92[a]	11·07	10·96	
6								14 20[c]	—					
7	8·75	8·65	16·97	21·08				17·53[a]	12·61[a]			12·26	12·21	
8	8·82	8·64	15·61	12·10	9·05			12·13	11·99			11·04	11·01	(1·18, 1·23)
9	11·04	10·74	23·81	22·32				11·97[c]	—			12·72	12·72	
10	8·37	8·54	71·61[b]	67·92[b]		39·98[c]	—					10·63[a]	10·00[a]	
11	8·72	8·17	38·59[b]	40·16[b]		8·97	8·53	12·15	12·31	1·96	1·91	10·86	10·97	
12	8·68	8·79	6·52	8·47				11·28	11·12			11·24	11·24	
13	8·62	7·78	58·22[a]	42·91[a]		9·54	9·62	10·56	10·28			11·99	11·83	
14	10·85	11·52										11·61	11·23	
15	10·14	9·91	13·43	13·36		11·00	10·26	10·55	10·44	2·03	2·05	10·86	10·90	
16	9·71	9·88	14·26	15·93				11·61	11·88			11·83	12·02	
17	8·67	9·02	9·12	13·53		9·19[a]	7·07[a]	5·99[b]	5·60[b]	1·57	1·58	12·28	12·28	(0·47, 0·57)
18	9·52	9·81	19·45	20·73		9·53	9·88	12·05	11·61			11·42	11·43	
19	8·81	8·30	31·14[a]	14·69[a]	6·80	7·97	8·63	15·25	14·37	2·68	2·76	11·62	11·85	(2·38, 2·33)
20	8·00	9·03	17·99	16·43	3·64							12·09	12·14	(0·16, 0·69)
21	9·26	9·60						14·05	14·72			11·54[c]	—	
22	9·62	9·38	10·25	11·28	10·57	9·43	10·16	11·87	11·83	1·98	1·93	12·65	12·57	(2·07, 1·71)
Mean $\bar{x}$	9·31		14·44		7·93	9·31		12·07		2·11		11·79		—
Repeatability r	0·85		4·74		—	1·20		1·03		0·09		0·29		—
Reproducibility R	2·49		14·03		—	2·66		3·98		1·32		1·73		—

For key, see Table VIII.

TABLE III

DIETARY FIBRE COLLABORATIVE TRIAL RESULTS
WHOLEMEAL DIGESTIVE BISCUITS

Laboratory	Dietary fibre g/100 g (d. wt basis) AACC method		MWS method		Englyst method	ISO method		AOAC method		Crude fibre g/100 g (d. wt basis)		Moisture g/100 g		Lignin g/100 g
1	1·65	1·96	10·37[c]	—	2·44	1·00	0·89	6·87[c]	9·91[a]	0·45	0·50	3·42	3·39	(—)
2	1·53	1·72	2·86	2·43	3·08							2·60	2·88	(0·0)
3	7·59	7·27	8·00	7·72		4·12[a]	6·07[a]	22·47[a]	5·91[a]	1·25[b]	1·22[b]	3·67	3·71	
4					3·00									(—)
5	3·50[a]	5·75[a]								0·49	0·50	2·83	2·87	
6								6·60[c]	—					
7	1·45	1·65	23·55	23·03				5·77	3·08			3·06	3·06	
8	1·61	1·80	9·79	7·68	3·68			3·58	3·58			2·55	2·54	(0·25, 0·38)
9	4·84	5·08	20·59	14·99				6·61	—			3·17	3·14	
10	1·52	1·64	64·16[b]	66·23[b]		13·13[c]	—					2·49	2·62	
11	4·20	4·44	23·16	29·94		1·19	1·76	4·90	5·07	0·40	0·37	3·17	3·34	
12	3·96	4·07	21·89	20·29				2·65	2·57			4·16	4·12	
13	2·16	1·43	34·97	31·29		1·32	1·23	5·12	4·23			3·02	3·05	
14	4·82	4·80	24·28	23·55								3·47	3·77	
15	2·31	1·69	10·96	11·20		4·00[b]	4·28[b]	4·55	4·41	0·56	0·47	3·50	3·49	
16	1·94	1·50	20·43	19·12				5·36	5·37			2·85	2·89	
17	1·96	2·53	21·60	22·98		1·94	1·74	0·00	1·95	0·32	0·34	2·64	2·65	(0·00, 0·00)
18	1·95	2·36	10·51	10·72		1·66	1·49	6·10	3·83			4·31	4·06	
19	1·34	1·04	27·21	34·15	2·60	0·87	1·17	8·71	8·06	0·50	0·44	2·98	2·93	(1·39, 0·67)
20	2·60	2·71	18·97	21·06	2·46							3·13	3·23	(−0·23, −0·43)
21	1·82	2·09						4·89	7·36			3·15[c]	—	
22	2·07	2·22	2·33	3·12	4·42	0·95	0·95	6·15	5·93	0·64	0·57	3·21	3·18	(0·57, 0·65)
Mean $\bar{x}$	2·72		17·64		3·10	1·30		4·72		0·47		3·19		—
Repeatability r	0·69		6·24		—	0·54		2·81		0·11		0·25		—
Reproducibility R	4·63		26·89		—	1·05		5·59		0·26		1·36		—

For key, see Table VIII.

TABLE IV

DIETARY FIBRE COLLABORATIVE TRIAL RESULTS
CORNFLAKES SAMPLE

Laboratory	Dietary fibre *g/100 g* (d. wt basis)									Crude fibre *g/100 g* (d. wt basis)		Moisture *g/100 g*		Lignin *g/100 g*
	AACC method 1		MWS method		Englyst method	ISO method		AOAC method						
1	1·18	1·21	12·88[c]	—	4·44	0·23	0·20	2·97[c]	—	0·35	0·34	5·32	5·26	(—)
2	0·91	1·04	1·87	3·43	4·10							4·86	5·06	(1·46)
3	3·71	3·56	8·03	5·29		1·48	1·26	4·87	6·32	0·79	0·64	4·96	4·98	
4					3·96									(—)
5	1·25	0·78								0·28	0·25	4·66	4·65	
6								7·29[c]	—					
7	0·75	0·00	9·77	13·10				5·61	4·43			4·96	4·99	
8	0·31	0·28	3·90	1·69	4·26			4·91	5·08			4·42	4·41	(0·62, 0·39)
9	2·67	2·78	16·46	14·70				4·79[c]	—			5·01	4·99	
10	1·27	1·19	77·18	77·98		10·53[c]						4·38	4·40	
11	0·35	0·66	15·19	17·06		0·30	0·42	3·96	3·97	0·31	0·32	5·10	5·02	
12	3·90	4·00	4·66	4·98				3·03	2·35			5·06	5·08	
13	0·30	0·65	51·07	50·86		0·24	0·24	3·25	2·81			5·08	5·09	
14	3·39	3·09										5·49	5·70	
15	1·96	1·06	6·65	7·42		2·55	2·48	2·60	2·27	0·56	0·53	4·75	4·82	
16	1·79	1·11	7·48	7·30				4·11	4·35			4·93	5·02	
17	1·02	1·32	0·00	2·80		2·19[a]	3·92[a]			0·31	0·32	4·35	4·37	(0·00, 0·00)
18	1·89	2·10	15·08	14·90		0·20	0·23	3·39	4·41			5·51	5·57	
19	0·74	0·91	78·94	73·78	5·33	0·02	0·07	6·34	7·12	0·72	0·47	5·08	4·96	(0·93, 1·01)
20	0·43	0·29	15·75	10·80	4·03							4·99	5·09	(−0·34, 0·03)
21	1·35	1·13						6·68[a]	9·88[a]			4·91[c]	—	
22	0·65	0·78	5·38	4·27	4·64	2·95[a]	3·46[a]	4·92	4·62	0·32	0·36	5·14	5·20	(0·39, 0·42)
Mean $\bar{x}$	1·44		19·62		4·39	0·71		4·31		0·43		4·97		—
Repeatability r	0·73		4·85			0·20		1·49		0·21		0·17		—
Reproducibility R	3·17		71·40			2·58		3·81		0·49		0·96		—

For key, see Table VIII.

TABLE V

DIETARY FIBRE COLLABORATIVE TRIAL RESULTS

BRAN SAMPLE

Laboratory	Dietary fibre *g/100 g* (d. wt basis) AACC method 1		MWS method		Englyst method	ISO method		AOAC method		Crude fibre *g/100 g* (d. wt basis)		Moisture *g/100 g*		Lignin *g/100 g*
1	50·53	49·88	52·94[c]	—	45·54	51·93	51·76	52·29[c]		10·12	9·66	11·15	11·18	(4·17)
2	48·27	49·49	53·46	53·12	33·22							10·37	10·68	(6·12)
3	52·50	56·12	36·60[a]	43·48[a]		32·76[a]	52·86[a]	58·72	59·33	11·61	11·63	10·92	10·94	
4					43·03									(—)
5	51·33	48·08								10·13[a]	13·26[a]	9·89	9·98	
6								56·58[c]	—					
7	49·10	48·45	53·04	54·10				46·17[a]	75·23[a]			10·92	11·07	
8	47·07	47·58	54·42	52·03	39·18			49·63	51·77			11·08	11·10	(5·47, 5·08)
9	49·91	50·98	56·29	59·70				52·38[c]	—			11·20	11·21	
10	45·73	45·81	65·49	64·85		55·30[c]	0·0					8·09	7·45	
11	49·15	49·32	51·10	51·73		53·72	53·12	55·36	54·79	10·46	10·59	9·41	9·57	
12	48·15	47·71	57·21	59·29				53·88	53·90			9·42	9·50	
13	47·53	47·64	63·44	60·45		50·70	50·44	52·22	53·64			10·02	9·89	
14	51·71	50·63										10·39	9·92	
15	46·69	47·07	52·91	51·65		53·74	53·11	50·71	51·48	11·44	11·28	9·12	9·21	
16	49·27	48·85	52·47	52·59				46·07	46·59			10·59	10·66	
17	48·00	47·65	49·49	50·57		50·01[a]	52·35[a]			8·38	8·56	10·81	10·87	(4·14, 4·50)
18	49·75	52·40	55·84	57·00		51·58	50·88	52·23	54·49			9·60	9·87	
19	42·74[c]	—	34·21[a]	43·68[a]	38·32	49·29[a]	55·68[a]	46·81[a]	57·27[a]	16·41[b]	15·34[b]	9·54	9·52	(9·64, 8·77)
20	48·42	48·05	51·82	52·99	22·19							10·48	10·58	(3·88, 2·65)
21	50·14	49·77						54·76	56·58			9·20[c]	—	
22	48·95	48·56	52·32	50·69	35·38	50·20	49·46	53·71	53·30	11·33	10·91	10·83	10·82	(8·46, 8·20)
Mean $\bar{x}$	49·11		55·00		36·41	51·72		53·16		10·50		10·21		—
Repeatability r	2·78		3·41		—	1·12		2·59		0·56		0·44		—
Reproducibility R	5·72		12·67		—	4·26		9·70		3·34		2·51		—

For key, see Table VIII.

TABLE VI

DIETARY FIBRE COLLABORATIVE TRIAL RESULTS

INSTANT PORRIDGE CEREAL SAMPLE

Laboratory	Dietary fibre *g/100 g* (d. wt basis)									Crude fibre *g/100 g* (d. wt basis)		Moisture *g/100 g*		Lignin *g/100 g*
	AACC method		MWS method		Englyst method	ISO method		AOAC method						
1	4·42	4·45	14·66[c]	—	7·31	3·44	3·47	8·86[c]	—	1·04	1·03	7·93	7·94	(0·43)
2	4·14	4·26	6·40	5·62	5·69							6·72	6·77	(1·71)
3	7·54[a]	6·30[a]	1·71	5·32		6·34[a]	4·51[a]	5·34	10·16	1·19	0·97	8·24	8·20	
4					7·09									(—)
5	5·67	5·19								0·68	0·90	7·01	7·04	
6								9·76[c]	—					
7	3·25	3·10	19·29	18·37				12·60	13·15			7·44	7·58	
8	3·78	4·36	21·65	18·58	6·39			11·93	13·16			6·30	6·34	(1·15, 1·17)
9	6·89	7·11	15·03	20·17				13·69[c]	—			8·05	8·03	
10	4·02	3·96	72·99[b]	74·11[b]		18·96[c]	—					6·13[a]	5·60[a]	
11	5·82	6·28	15·47	14·50		3·85	3·90	12·28	12·86	0·89	0·86	7·06	7·01	
12	3·68	3·57	7·26	9·72				11·55	12·60			7·64	7·70	
13	2·67	2·87	56·38[b]	50·98[b]		3·09	3·06	10·71	10·91			7·22	7·21	
14	6·27	6·27	33·33	38·11								7·19	7·43	
15	4·98	5·05	11·43	13·39		5·61	5·81	5·79	5·57	1·09	1·00	7·19	7·03	
16	4·39	4·39	11·20	11·09				13·16	12·99			7·05	7·11	
17	3·72	3·03	11·46	16·85		2·32[a]	7·02[a]			0·68	0·65	7·69	7·70	(0·32, 0·53)
18	4·08	3·40	19·55	20·57		3·08	3·33	8·79	10·38			7·50	7·34	
19	3·15	3·10	79·30[a]	65·68[a]	5·34	3·38[a]	2·54[a]	11·81	13·28	1·36	1·52	7·19	7·23	(2·00, 1·68)
20	4·10	4·07	17·71	20·61	2·48							7·60	7·62	(0·50, 0·31)
21	4·45	4·54						10·81	5·39					
22	3·78	4·03	8·20	10·41	12·46	4·01	4·33	10·28	10·65	0·83	0·77	7·38[c]	7·74	(1·25, 1·30)
Mean $\bar{x}$	4·38		15·11		6·68	3·92		10·67		0·97		7·38		—
Repeatability r	0·64		6·03		—	0·37		4·50		0·26		0·18		—
Reproducibility R	3·23		22·99		—	2·76		7·55		0·71		1·34		—

For key, see Table VIII.

TABLE VII

DIETARY FIBRE COLLABORATIVE TRIAL RESULTS

SOYA FLOUR SAMPLE

Laboratory	Dietary fibre *g/100 g* (d. wt basis)									Crude fibre *g/100 g* (d. wt basis)		Moisture *g/100 g*		Lignin *g/100 g*
	AACC method		MWS method		Englyst method	ISO method		AOAC method						
1	5·11	5·07	21·49[c]	—	10·38	0·47	0·62	10·86[c]	—	2·01	2·05	5·88	5·92	(0·19)
2	3·83	3·65	14·11	13·79	9·02							4·83	4·65	(3·10)
3	6·42	7·32	20·72	23·60		2·37[c]	—	33·52	34·38	2·62	2·39	6·18	6·21	
4					10·47									(—)
5	6·46[a]	8·07[a]								1·59	1·38	5·21	5·22	
6								18·85	—					
7	4·85	3·80	33·54	34·71				22·15	24·87			5·15[a]	5·74[a]	
8														
9	6·12	6·20	34·02	31·24				7·04[c]	—			5·92	5·88	
10	4·42	4·72	35·46	40·52		1·53						3·73[a]	3·05[a]	
11	6·57	6·50	19·09	25·16		0·70	0·88	26·66	24·26	2·31	2·28	5·04	5·10	
12	4·05	4·16	14·97	17·66				16·74	16·73			6·30	6·16	
13	2·93	3·35	28·30	31·90		0·49	0·54	13·67	15·28			5·29	5·16	
14	6·18	5·85	18·21	18·72								5·98	6·27	
15	5·76	5·22	27·64	26·47		3·82	4·57	23·75	25·06	2·09	2·17	5·80	5·81	
16	5·57	6·08	35·73	33·93				23·57	24·93			5·41	5·65	
17	3·75	3·12	21·57	24·17		0·28	0·71	15·54	16·47	1·66	1·58	5·44	5·41	(0·35, 0·42)
18	4·75	4·69	17·82	17·38		0·78	0·57	13·67	9·87			5·92	5·84	
19	3·54	3·62	32·01	31·56	7·64	<0·01[d]	0·02[d]	18·19[a]	24·32[a]	2·30[a]	2·91[a]	5·50	5·59	(0·92, 1·06)
20	4·87	4·86	29·46	27·64	3·66							5·73	5·76	(1·48, 1·03)
21	3·96	4·25						12·25	12·72			5·41[c]	—	
22	4·40	4·13	30·79	28·44	9·10	3·75[b]	4·20[b]	10·73	10·97	1·93	1·97	5·53	5·69	(1·24, 1·44)
Mean $\bar{x}$	4·82		26·26		8·38	1·20		19·45		2·00		5·63		—
Repeatability r	0·88		5·50		—	0·75		3·60		0·26		0·26		—
Reproducibility R	3·20		21·11		—	4·19		20·86		1·03		1·21		—

For key, see Table VIII.

TABLE VIII

KEY TO TABLES I–VII

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

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

c. one result only reported. Not included in the calculation of mean, repeatability and reproducibility.

d. approximate value. Pair not included in the calculation of mean, repeatability and reproducibility.

e. in the Englyst procedure's results, the value quoted included resistant starch as not all the laboratories participating in the trial carried out the complete procedure to obtain the RS value. As may be seen from Table X, the values for RS are very small with the exception of that for the cornflakes samples.

procedure is combined with Van Soest's Acid Detergent Fibre and lignin procedures, values may be obtained for hemicellulose, cellulose and lignin[30].

A disadvantage of the procedure is loss of water-soluble cell wall polysaccharides, which in some foods such as vegetables may be 40–60 per cent. of total dietary fibre. More than 50 per cent. of NSP from white bread and oats, and around one third in whole wheat, is solubilised at pH 7[24], and therefore will be lost in the NDF (and AACC)[30] procedure. Loss of water-soluble polysaccharides will therefore lead to an underestimation of NSP whilst inadequate removal of starch and/or protein and inclusion of substances measuring as lignin will tend to have the opposite effect on the final value[43].

Complete removal of starch from white flour is known to be difficult[24] but the mean value of NSP of 1·16 per cent. obtained by Van Soest's procedure corresponds well to insoluble NSP measured in the Englyst procedure[27] (Table X). This indicates that starch and protein have been completely removed but, as expected, the whole of the water-soluble fraction, which is 64 per cent. of total NSP in white flour, is lost in Van Soest's NDF procedure.

For wheat bran, which contains only a small amount of soluble material (Table X), good agreement is obtained between Van Soest's, the AOAC procedure and Englyst's procedure when lignin is taken into account.

Cornflakes have 0·5 per cent. of insoluble NSP and 3·2 per cent. of RS (Table X). Assuming that insoluble NSP is totally recovered in Van Soest's procedure and that there are only traces of lignin present in cornflakes, RS has been about 70 per cent. solubilised and removed in the Van Soest procedure.

The 4·8 per cent. of NDF measured for soya flour agrees with the 5·1 per cent. of insoluble NSP measured by the Englyst procedure (Table X). This is an indication that the large amount of protein in soya has been removed completely by Van Soest's procedure. Van Soest's NDF method aims to measure insoluble dietary fibre and the study as a whole indicates that this is adequately done.

No special difficulties were reported by the participants.

AN MWS "SIMPLE" METHOD FOR "DIETARY FIBRE"

This is a development of the unavailable carbohydrate method described in 1936 by McCance, Widdowson and Shackleton[44]. It is a "by difference" method, determining the remainder after starch, protein and ash have been measured in an ethanol insoluble fraction. The foodstuff is first treated with 80 per cent.

TABLE IX

SUMMARY OF DIETARY FIBRE RESULTS OBTAINED IN COLLABORATIVE TRIAL

Sample	AACC	MWS	Dietary fibre method Englyst		ISO	AOAC
			NSP + RS	*RS*		
White flour						
No. of laboratories*	19	17	7		10	16
Mean ($\bar{x}$)†	1·16	12·55	3·47	0·25	0·82	3·83
Repeatability (r)†	0·51	4·51	—		0·35	0·64
Reproducibility (R)†	1·36	28·70	—		2·04	2·68
Standard deviation	—	—	0·73		—	—
Added bran flour						
No of laboratories	20	17	7		10	16
Mean ($\bar{x}$)	9·31	14·44	7·93	0·22	9·31	12·07
Repeatability (r)	0·85	4·74	—		1·20	1·03
Reproducibility (R)	2·49	14·03	—		2·66	3·98
Standard deviation	—	—	2·28		—	—
Wholemeal digestive biscuits						
No. of laboratories	20	18	7		10	16
Mean ($\bar{x}$)	2·72	17·64	3·10	0·17	1·30	4·72
Repeatability (r)	0·69	6·24	—		0·54	2·81
Reproducibility (R)	4·63	26·89	—		1·05	5·59
Standard deviation	—	—	0·73		—	—
Cornflakes						
No. of laboratories	20	17	7		10	15
Mean ($\bar{x}$)	1·44	19·62	4·39	3·23	0·71	4·31
Repeatability (r)	0·73	4·85	—		0·20	1·49
Reproducibility (R)	3·17	71·40	—		2·58	3·81
Standard deviation	—	—	0·48		—	—
Bran						
No. of laboratories	20	17	7		10	14
Mean ($\bar{x}$)	49·11	55·00	36·41	0·03	51·72	53·16
Repeatability (r)	2·78	3·41	—		1·12	2·59
Reproducibility (R)	5·72	12·67	—		4·26	9·70
Standard deviation	—	—	7·30		—	—
Instant porridge cereal						
No. of laboratories	20	18	7		10	15
Mean ($\bar{x}$)	4·38	15·11	6·68	0·10	3·92	10·67
Repeatability (r)	0·64	6·03	—		0·37	4·50
Reproducibility (R)	3·23	22·99	—		2·76	7·55
Standard deviation	—	—	3·01		—	—
Soya flour						
No. of laboratories	19	17	6		10	15
Mean ($\bar{x}$)	4·82	26·26	8·38	0·00	1·20	19·45
Repeatability (r)	0·88	5·50	—		0·75	3·60
Reproducibility (R)	3·20	21·11	—		4·19	20·86
Standard deviation	—	—	2·53		—	—

* Total number of laboratories carrying out method. (For each sample given.)
† Values calculated after rejection of outliers. (For each sample given.)

ethanol to remove soluble sugars etc. and the residue recovered. Starch, nitrogen and ash are then determined separately in the residue and, after appropriate conversion factors have been applied these values are subtracted from the ethanol insoluble fraction. The result is a value for dietary fibre which

TABLE X

NON-STARCH POLYSACCHARIDES (NSP) AND RESISTANT STARCH (RS) IN THE 7 MAFF TEST SAMPLES: (ENGLYST PROCEDURE)

	Sample *g/100 g dry matter*						
	A White flour	B Flour + bran	C Biscuits	D Cornflakes	E Wheat bran	F Oats	G Soya flour
Soluble NCP							
Arabinose	0·66	0·76	0·42	0·07	0·28	0·21	0·87
Xylose	0·86	1·07	0·63	0·07	0·13	0·17	0·25
Mannose	0·01	0·04	0·03	t	0·01	—	0·13
Galactose	0·17	0·19	0·13	t	0·13	0·08	1·63
Glucose	0·12	0·22	0·14	0·05	—	3·56	—
Uronic acids	0·05	0·03	0·01	0·03	0·23	0·10	0·62
Total	1·87	2·31	1·36	0·22	0·78	4·12	3·50
Insoluble NCP							
Arabinose	0·28	1·72	0·38	0·09	10·15	0·63	1·04
Xylose	0·47	2·96	0·62	0·12	17·38	1·02	0·51
Mannose	0·05	0·12	0·03	t	0·24	0·07	0·39
Galactose	t	0·12	t	t	0·61	0·09	2·21
Glucose	0·12	0·62	0·12	—	3·31	0·59	—
Uronic acids	t	0·21	0·03	0·03	1·15	0·16	0·91
Total	0·92	5·75	1·18	0·24	32·84	2·56	5·06
Cellulose							
Glucose	0·18	1·54	0·31	0·26	9·62	0·35	1·93
Total NSP	2·97	9·60	2·85	0·72	43·24	7·03	10·49
Resistant starch	0·25	0·22	0·17	3·23	0·03	0·10	—

Values obtained by a laboratory experienced in using this procedure.

includes lignin and other insoluble substances. The method is given in detail in Appendix I.

In the method starch is only partly hydrolysed to glucose by the starch removal procedure and therefore will be partly included in the dietary fibre value. On the other hand β-glucans such as are found in oats, barley and rye and which are a legitimate component of NSP will be largely hydrolysed by the 2-h treatment with 0·75 mol/1 H_2SO_4 and therefore lost. A further source of error is the factor used to convert nitrogen to protein. This factor will depend on the actual foodstuff being analysed and will not be available in unknown samples. Lack of precision in the measurement of protein and starch, particularly in foods where these represent the greater proportion of the dry weight, will lead to large errors in the calculated value for unavailable carbohydrate and lignin. In the present trial much higher values were obtained for dietary fibre by the MWS procedure than by any other method used in the trial, mainly due to the considerable difficulties participants had with starch removal and the estimation of protein.

DIETARY FIBRE MEASURED AS NON-STARCH POLYSACCHARIDES; ENGLYST PROCEDURE[24]

This method measures dietary fibre as the non-starch polysaccharides (NSP) in plant foods. Starch is removed enzymatically and NSP measured as the sum of constituent sugars released by acid hydrolysis.

In the method, two food samples are heated with acetate buffer for 2 h at 100°C and then incubated overnight at 40°C with an excess of hog pancreatic α-amylase and pullulanase. Starch which resists hydrolysis (mainly due to retrogradation during food processing) is redispersed with KOH, degraded enzymatically and measured as a fraction called Resistant Starch (RS)[24]. This measurement of RS is applied as a pretreatment to procedure B (see below).

Following removal of the non-resistant starch the two samples are analysed by separate but complementary procedures: (A) Hydrolysis with 1 mol/1 H_2SO_4 after dispersion of cellulose with 12 mol/1 H_2SO_4; and (B) Direct hydrolysis with 1 mol/1 H_2SO_4. Released neutral sugars are measured by GLC as alditol acetates, and uronic acids by a colorimetric method[45].

Procedure A gives a value for total NSP (after subtraction of RS) and Procedure B a value for neutral non-cellulosic polysaccharides (NCP) (again after subtraction of RS). A value for cellulose is obtained by subtracting the value for glucose in the NCP from that obtained for NSP in procedure A.

If raw products are analysed, the value for NSP obtained by procedure A is correct. However, when analysing processed foods RS will, as for all other methods described in this paper, interfere and invalidate the results in procedure A. Only if RS is measured separately (by the pretreatment to procedure B), and then subtracted from the NSP value in procedure A, can a true value for NSP be obtained. (The separate measurement of soluble-NSP was not part of the trial but values are included in Table XIII because of their use in explaining the difference between results obtained by various methods.)

This method gives results which are similar to those of detailed structural studies of cell-wall material[46] and for NSP components other than glucose and uronic acids similar to those of the method of Selvendran[37] (see Table XI).

The uronic acid values obtained for similar products by Theander's decarboxylation procedure[25], which is thought to be the most accurate method, agree well with those obtained in the Englyst procedure, being zero for white flour, 1·4 per cent.[25] for wheat bran, and 1·1 per cent. for soya flour with 8·7 per cent. of NCP.

The results obtained by participants in the present trial suffer to some extent from lack of suitable equipment which did not allow the procedure to be followed accurately.

Modifications introduced by some analysts included the use of other than specified GLC columns and conditions, use of tubes other than the screw-cap glass centrifuge tubes, and samples kept at unsuitable stages for prolonged time periods due to lack of a multi-evaporator.

The lower values obtained by laboratory 20 may be related to the dispersion of cellulose at 45°C for an unspecified time period and not for 1 h at 35°C as given in the procedure.

Only a few participants have measured RS separately and the values in Tables

TABLE XI

COMPARISON OF RESULTS OBTAINED BY THE METHODS OF ENGLYST AND OF SELVENDRAN

Sample	Procedure[a]	Rha + Fuc	Ara	Xyl	Man	Gal	Glu	Uronic acids	Total NSP	RS
White flour	S	0·02	0·98	1·35	0·12	0·19	3·04	0·33	6·04	
	E	t	0·94	1·33	0·06	0·17	0·42	0·05	2·97	0·25
Added bran flour	S	0·03	2·68	3·85	0·16	0·33	4·64	1·06	12·75	
	E	t	2·48	4·03	0·16	0·31	2·38	0·24	9·60	0·22
Wholemeal digestive biscuits	S	0·01	0·85	1·22	0·08	0·15	1·91	0·32	4·54	
	E	t	0·80	1·25	0·06	0·13	0·57	0·04	2·85	0·17
Cornflakes	S	0·01	0·21	0·22	0·02	0·02	5·22	0·81	6·52	
	E	t	0·16	0·19	t	t	0·31	0·06	0·72	3·23
Wheat bran	S	0·12	11·06	17·31	0·45	0·86	14·20	3·61	47·59	
	E	t	10·43	17·51	0·25	0·74	12·93	1·38	43·24	0·03
Instant porridge oats	S	0·05	1·18	1·33	0·20	0·24	6·71	0·89	10·61	
	E	t	0·84	1·19	0·07	0·17	4·50	0·26	7·03	0·10
Soya flour	S	0·56	1·97	0·74	0·56	3·49	2·41	3·70	13·44	
	E	0·63	1·91	0·76	0·52	3·84	1·93	1·53	11·12	0·00

Notes

[a] Methods used those of Englyst *et al.* (ref. 24) (E) and of Selvendran *et al.* (ref. 37) (S).
[b] Both methods used by laboratories experienced with the procedures.
[c] Samples used in the comparison are the trial samples.
[d] All results as g/100 g, dry matter basis.
[e] Key: Rha, Rhamnose; Fuc, Fucose; Glu, Glucose; Ara, Arabinose; Xyl, Xylose; NSP, Non-starch polysaccharide; Man, Mannose; Gal, Galactose; Rs, Resistant starch.

III to VIII therefore include RS.

Values obtained by this procedure are considerably lower than those obtained by the other trial procedures aiming to measure total dietary fibre. The main reason is that starch is completely removed and that substances measuring as lignin are not included.

A further development of this method has now been made[28], since the initial trial was carried out, in which direct dispersion and removal of all starch including resistant starch is achieved allowing for a more rapid determination of total NSP in foods. This method will be the subject of a further report of this collaborative trial.

The exact analytical procedure used in the initial trial is given in Appendix II.

ISO PROCEDURE FOR THE DETERMINATION OF BRAN BY AN ENZYMIC METHOD[31]

This is an ICC procedure taken by the ISO Sub-Committee ISO/TC 34/SC4 (Cereals and Pulses) as a method for the determination of bran content in cereals. The latest draft of the method, at the time of the trial, was used and is based on the grinding of the sample, defatting by an acetone/ether mixture, forming a paste and treating in subsequent stages with bacterial α-amylase and alkaline protease agents to remove starch and protein. After each hydrolysis, the residue is thoroughly washed with tap water. The whole procedure is

repeated and a final acetone treatment included. A value for fibre is obtained as the weight of residue.

Starch and protein appear to be completely removed by this procedure but so also is a high proportion of the non-starch polysaccharides. Hot water extraction at alkaline pH is known to solubilize large quantities of non-cellulosic polysaccharide[24]. To this extent the method is similar to the neutral detergent fibre procedure of Van Soest. However it is more complicated and less precise than the Van Soest procedure.

"AOAC" PROCEDURE FOR THE DETERMINATION OF DIETARY FIBRE[22]

At the time of the first MAFF collaborative trial an "AOAC" method was being subjected to a parallel trial, the results of which are now in press[22].

This is the most recently reported method for measuring dietary fibre and is a combination of enzymic and gravimetric procedures based on the work of Asp *et al.*[20], Furda *et al.*[47] and Schweizer and Wursch[21]. Its object is to measure "the sum of the soluble and insoluble polysaccharides and lignin". In principle the method involves an initial defatting of the dried food samples followed by starch gelatinisation and incubation with heat-stable α-amylase Termamyl. Further digestion is then accomplished with amyloglucosidase and protease. Soluble fibre is precipitated with 95 per cent. ethanol and the residue washed with ethanol, acetone, then dried and weighed. Protein is measured on the final residue and it is ashed. Dietary fibre equals the weight of residue less residual protein (N × a factor) and ash.

TABLE XII

DIETARY FIBRE CONTENTS OF SAMPLES USED IN AOAC COLLABORATIVE STUDY DETERMINED BY "AOAC" PROCEDURE AND ENGLYST PROCEDURE

Sample	Dietary fibre—AOAC procedure *g/100 g*	Dietary fibre—Englyst procedure *g/100 g*	AOAC/Englyst ratio
Corn bran	89·02	78·48	1·13
Iceberg lettuce	23·31	19·24	1·21
Oats	12·47	7·59	1·64
Instant potatoes	7·22	4·74	1·52
Raisins	4·43	1·89	2·34
Rice	3·67	0·62	5·91
Rye bread	5·90	3·17	1·86
Soy isolate	7·51	0·97	7·74
Wheat bran	42·25	32·21	1·31
Whole wheat flour	12·92	9·79	1·31
White wheat flour	3·07	1·83	1·67
Mixed diet, non-veget	7·19	4·11	1·74
Mixed diet, veget	8·59	4·96	1·73

Notes

[a] Methods used those of "AOAC" (ref. 22) and of Englyst *et al.* (ref. 24).

[b] Dietary fibre by the AOAC procedure are the mean of values obtained by participants in the AOAC trial. Values by the Englyst method are from one laboratory experienced with the procedure.

[c] Samples used in the comparison are the AOAC trial samples.

[d] All results as g/100 g, dry matter basis.

TABLE XIII

COMPARISON OF DIETARY FIBRE VALUES OBTAINED BY THE METHODS OF ENGLYST AND OF SOUTHGATE

Sample	Southgate		Englyst
	Dietary fibre	Lignin	Dietary fibre
White flour	5·40	0·28	2·97
Added-bran flour	11·05	1·18	9·60
Wholemeal digestive biscuits	3·59	0·58	2·85
Cornflakes	4·80	1·74	0·72
Wheat bran	41·43	4·89	43·24
Instant porridge oats	8·95	1·42	7·03
Soya flour	10·61	1·32	11·12

Notes

a. Methods used those of Englyst *et al.* (ref. 24) and of Southgate (ref. 35).
b. Both methods used by different laboratories experienced with the procedures.
c. Samples used in the comparison are the trial samples.
d. Dietary fibre values do not include lignin.
e. All results as g/100 g, dry matter basis.

The method has recently undergone extensive international trials in the USA and Europe[22]. As with all gravimetric methods, however, it needs to be validated against a more detailed chemical method such as that of Englyst *et al.*[24] or Theander and Aman[25]. The values obtained in the trial were higher than those of more detailed methods (see Table XII). This is probably due to a combination of factors. The final residue contains substances other than cellwall polysaccharides such as lignin, cutin, suberin, tannin, Maillard products etc. The incorrect choice of the protein factor in the correction of the residue for its protein content may also lead to errors. In addition, some resistant starch is probably included in the residue. All of these problems will tend to lead to an overestimate of NSP especially in foods where NSP is only a small proportion of total solids such as white bread, potatoes, rice etc. It is a relatively complex and tedious method which offers no advantages in time over more detailed and precise analytical procedures which are likely to prove as good if not more robust in general analytical laboratories.

Values for cornflakes (Table IV) by the AOAC procedure are considerably higher than the NSP values by Englyst's procedure but lower than for NSP + RS. Taking lignin measuring substances and the high variation into account, this may indicate that the AOAC procedure does not remove all, but rather a variable unspecified amount, of the material measuring as RS in Englyst's procedure.

The 19·45 per cent. of dietary fibre in soya flour compared with 8·38 per cent. of NSP (Table IX), together with the very high variation, indicate considerable difficulties with removal and correction for protein in the AOAC procedure. The incomplete removal of, or correction for, protein, partial removal of RS, and the inclusion in the AOAC procedure of substances measuring as lignin, seem to be the main reasons for the difference between values by the AOAC procedure and the NSP values obtained using the procedure by Englyst *et al.*[24]

The AOAC procedure includes soluble NSP and values are, for all products,

higher than for insoluble NDF by Van Soest's procedure. Reproducibility is similar by the two procedures.

Overall, it will be seen that most of the values of repeatability and reproducibility are unacceptably high for any standardised analytical method. The values approach or, in the case of the reproducibilities sometimes exceed, the mean value of dietary fibre in the sample being analysed.

In addition none of the methods may be said to be significantly better in terms of analytical performance than any other though, even given that only a limited number of laboratories undertook the Englyst procedure, it would appear that that method may be most promising.

It will be seen that the results of the lignin determinations are very variable and thus suggest that it is appropriate not to include this determination in any definition of dietary fibre for analytical reasons as well as those given previously.

Conclusions

The results of the initial collaborative trial were disappointing and it was not possible to recommend any specific method for the determination of dietary fibre.

The Advisory Panel considered the results and recommended that, as the Englyst method appeared to be the most accurate and informative, it should be further studied, with a view to simplifying the procedure if possible, and once simplified, it should be re-tested. In addition it was decided that the determination of lignin should not be included in any method for the estimation of dietary fibre.

The Englyst method was simplified and the simplified method has now been tested collaboratively. The results of that trial are to be reported in the second part of this Report.

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Appendix I: Simple MWS Procedure for the Determination of Total Dietary Fibre

1. SCOPE AND FIELD OF APPLICATION

The method determines total dietary fibre in foodstuffs.

2. DEFINITION

The total dietary fibre: the total dietary fibre content as determined by the method specified.

3. PRINCIPLE

The portion of food insoluble in 80 per cent v/v ethanol is prepared. This alcohol insoluble residue (AIR) is analysed for starch, total nitrogen, moisture and ash. Total dietary fibre is calculated by subtracting the sum of starch, protein (total Nx 5·7), moisture and ash from the weight of AIR.

4. REAGENTS

All reagents should be of analytical quality reagent grade, and distilled water or water of equivalent purity is to be used.

4.1 *Ethanol*

95 per cent. v/v. Dilute with water as appropriate.

4.2 *Acetone*

5. APPARATUS

5.1 Flasks, 250 ml round bottom fitted with air cooled condenser.

5.2 Water bath, boiling.

6. PROCEDURE

6.1 *Sample Preparation*

Thoroughly mix and grind or homogenise the sample so that it passes through a 1-mm sieve.

Determine the moisture content on the prepared sample using the oven drying procedure.

6.2 *Preparation of Alcohol Insoluble Residue (AIR)*

Weigh out an amount of sample equivalent to 5–10 g of dry matter in a tall 250-ml beaker. Add 50 ml of aqueous ethanol (4.1) to bring the final concentration to 80 per cent. v/v of ethanol. With foods of high moisture content the preparation may have to be changed accordingly. Bring to the boil with

continuous stirring, remove from the heat and allow the solids to settle for a few minutes. Decant most of the ethanol through a Whatman 541 filter paper. Repeat the extraction 3 times with 25 ml of 80 per cent. v/v ethanol (from reagent 4·1), allowing the filter to drain between each extraction. If the free sugars are to be measured, retain all the ethanolic filtrate and make to volume. Finally, rinse the extracted solids in the beaker with 50 ml of warm acetone and decant through the filter. Allow the solids in the beaker and the filter paper to dry in air. Return any fine particles retained on the filter to the beaker by brushing off with a soft brush when the filter paper is dry.

Once the AIR has been prepared, allow it to equilibrate with air, weigh, grind up gently in a mortar to a fine, even powder and store in an airtight container.

6.3 *Analysis of the Alcohol Insoluble Residue*

On a portion of the AIR, prepared as in 6.2, determine:

6.3.1 *Moisture Content of AIR*

Use the moisture method specified, i.e. by oven drying at 103 ± 2°C.

6.3.2 *Total Nitrogen Content of AIR*

Use a standard Kjeldahl procedure. Protein is N × 5·7.

6.3.3 *Ash Content of AIR*

Ignite a portion of AIR in a silica crucible at 475 ± 25°C.

6.3.4 *Starch Content of AIR*

Accurately weigh approximately 100 mg of AIR into a quickfit stoppered tube, BC24/C24R. Add a small magnetic stirrer bar and 4 ml of water at 70°C. Stopper the tube loosely, heat on a stirrer hot plate and boil for 10 min to gelatinise the starch.

Note: steam condenses on the cold sides of the tube and there is no loss of volume.

Remove the stopper and cool in water to room temperature. Quantitatively transfer the contents of the tube to a 250-ml round bottom flask with 75 ml of 0·75 mol/l sulphuric acid and fit on an air-cooled condenser. Heat the flask in a boiling water bath or steam bath at 95–100°C for 2 h. It should not be necessary to police down any of the solids since the flask contents do not boil. After hydrolysis, cool the flask to room temperature in cold water and quantitatively transfer the contents (but not the stirrer bar) to a 100-ml graduated flask using 0·75 mol/l sulphuric acid. Make to the mark with dilute acid and mix well. Filter approximately 50 ml of hydrolysate through a Whatman 541 filter paper and collect the middle 30 ml. Pipette out 25 ml of this filtered aliquot into a 50-ml graduated flask and partially neutralise by the addition of 5 ml of 7·0 mol/l sodium hydroxide. Make up to volume with distilled water and mix well. Estimate the amount of glucose by a specific enzymic method (e.g. Bohringer Glucose Oxidase/Peroxidase Method).

7. EXPRESSION OF RESULTS

7.1 *Formulae and Method of Calculation*

The proportion, in g/100 g, of alcohol insoluble residue in the foodstuff as received is given by:

$$\frac{m_1 \times 100}{m_0}$$

where

m_1 is the mass in g of AIR obtained and stored at Section 6.2.

m_0 is the mass in g of sample taken to prepare the alcohol insoluble residue at Section 6.2.

The proportion, in g/100 g, of insoluble dietary fibre in the stored alcohol insoluble residue at Section 6.2 is given by:

$$100 - (\text{starch} + \text{protein} + \text{ash} + \text{water})$$

where

"starch" is the mass, in g/100 g, of starch in the prepared alcohol insoluble residue as determined at Section 6.3.4 (but note that starch = glucose × 0·9).

"protein" is the mass, in g/100 g, of protein in the prepared alcohol insoluble residue as determined at Section 6.3.2.

"ash" is the mass, in g/100 g, of ash in the prepared alcohol insoluble residue as determined at Section 6.3.3.

"water" is the mass, in g/100 g, of water in the prepared alcohol insoluble residue as determined at Section 6.3.1.

The proportion, in g/100 g, of total dietary fibre in the sample as received is given by:

$$\frac{m_1}{m_0}[100 - (\text{starch} + \text{protein} + \text{ash} + \text{water})]$$

where

$m_1 m_0$, starch, protein, water and ash are as calculated above.

7.2 *Repeatability*

The difference between the results of two determinations carried out simultaneously or in rapid succession on the same sample, by the same analyst, under the same conditions shall not exceed [ ] g per 100 g of sample. (The value will depend on the results of the collaborative trial.)

8. NOTES

The hydrolysis method used in Section 6.3.4 is a modification of the method of Pirt and Whelan; published in *J. Soc. Food Agric.*, 1951, **2**, 224–228. It will hydrolyse some of the cell wall components normally measured as dietary fibre, but they will not be included if the enzymic procedure is used to estimate glucose.

Appendix II: Englyst Procedure for Measurement of Non-starch Polysaccharides

1. SCOPE AND FIELD OF APPLICATION

The method described allows precise measurement of the principal fraction of dietary fibre, the non-starch polysaccharides (NSP) in plant foods. Modifications are described which enable a value to be obtained for cellulose and for starch resistant to digestion by α-amylase (resistant starch) which would otherwise be included as NSP.

2. DEFINITION

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

3. PRINCIPLE

The starch is gelatinised and then removed by enzymic digestion. The remaining polysaccharides are hydrolysed by sulphuric acid and the resulting individual neutral sugars are determined by gas-liquid chromatography (GLC) of the alditol–acetate derivatives and uronic acids by a colorimetric procedure and expressed as glucuronic acid (see Note 8.2).

4. REAGENTS

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

4.1 *Acetone*

4.2 *Ethanol, absolute*

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

4.3.1 *Sodium Acetate, 0·1 mol/l*

Prepare by dissolving 13·6 g of sodium acetate trihydrate, CH_3 COONa. $3H_2O$, in water saturated with benzoic acid, and make up to 1 litre with benzoic acid saturated water.

4.3.2 *Sodium Acetate Buffer, 0·1 mol/l*

Adjust sodium acetate, 0·1 mol/l (4.3.1) to pH 5·2 with acetic acid, 0·1 mol/l.

To stabilise and activate the enzymes add 4 ml of calcium chloride, 1 mol/l, to 1 litre of buffer (4·3).

4.4 *α-Amylase Enzyme*

Hog pancreas α-amylase, EC 3.2.1.1 (Sigma A 4268).

4.5 *Pullulanase Enzyme*

Pullulanase, EC 3.2.1.4.1 (Boehringer 108944)

4.6 *Mixed Enzyme Solution*

Solution containing 10 000 units of α-amylase (4.4) and 5 units of pullulanase (4.5) per ml of sodium acetate buffer (4.3.2). Prepare fresh immediately before use.

4.7 *Ethanol, 80 per cent. v/v*

4.8 *Sulphuric acid, 12 mol/l*

(reagent strength is critical and standardisation is required.)

4.9 *GLC Internal Standard Solution*

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

4.10 *Octan-2-ol*

4.11 *Barium Carbonate, $BaCO_3$*

Use as a fine powder.

4.12 *Potassium Hydroxide, 2 mol/l*

4.13 *Acetic Acid 2 mol/l*

4.14 *Amyloglucosidase Enzyme*

Amyloglucosidase EC 3.2.1.3 from Aspergillus niger NRRL (BDH 39114)

4.15 *Amyloglucosidase Solution*

Prepare by adding 0·2 ml of amyloglucosidase (4.14) to 5 ml of sodium acetate buffer pH 4·5 (4·16) and make up to 25 ml with water. Prepare fresh immediately before use.

4.16 *Sodium Acetate Buffer, 0·1 mol/l, pH 4·5*

Adjust sodium acetate, 0·1 mol/l (4.3.1) to pH 4·5 with acetic acid 0·1 mol/l. To stabilise and activate the enzymes, add 2 ml of calcium chloride, 1 mol/l, to 100 ml of buffer.

4.17 *Sulphuric acid, 2 mol/l*

4.18 *Mixed Resin*

Amberlite monobed resin MBI (BDH) treated with 1 mol/l ammonium carbonate until no more gas is developed, washed to neutrality with water and left as a slurry in a beaker.

4.19 *Ammonium Hydroxide, 3 mol/l*

4.20 *Ammonium Hydroxide/Sodium borohydride Solution*

A solution of 3 mol/l ammonium hydroxide containing 100 mg of sodium borohydride ($NaBH_4$) per ml.

4.21 *Methanol*

4.22 *Acetic Acid, glacial*

4.23 *Acetic anhydride*

4.24 *Sodium Chloride/Boric Acid Solution*

Add 2 g of sodium chloride, NaCl, and 3 g of boric acid, H_3BO_3, to 100 ml of water. Shake to dissolve.

4.25 *Sulphuric Acid, concentrated*

4.26 *Dimethylphenol Solution*

Add 0·1 g of 3,5 dimethylphenol, $(CH_3)_2\,C_6H_3.OH$, to 100 ml of glacial acetic acid (4.22). Shake to dissolve.

4.27 *Glucuronic Acid*

Solid, for preparation of standards used in uronic acid determination.

4.28 *Standard Sugar Solutions*

Prepare by weighing 1·000 g of each of the following sugars (dried to constant weight under vacuum with P_2O_5) into six separate 1-litre volumetric flasks:

L-rhamnose
L-arabinose
D-xylose
D-mannose
D-galactose
D-glucose

Dissolve the sugars in, and make up to volume with, benzoic acid saturated water. Take 10 ml of each sugar solution and mix with 10 ml of internal standard (4.9).

Also prepare a single standard solution containing 0·500 g/l of each of the above six standard sugars and 0·500 g/l of erythritol and 1·00 g/l of myoinositol.

Reduce and acetylate 2 ml of each of the standard solutions as described for the sample (see Sections 6.9.1.2 and 6.9.1.3).

5. APPARATUS

5.1 *Centrifuge*

Capable of at least 2 500 rpm.

5.2 *Centrifuge Tubes*

Glass centrifuge tubes of 50 ml capacity and fitted with screw-tops.

5.3 *Magnetic Stirrer Hot Plate*

The hot plate is fitted with a beaker of water. The temperature is variously

adjusted during the course of the procedure. It may be necessary to cover the beaker to maintain boiling.

5.4 *Magnetic Stirrer, PTFE-coated*

To fit centrifuge tubes (5.2)

5.5 *Evaporator*

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

5.6 *GLC Chromatograph*

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

5.7 *GLC Column and Conditions*

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

2·1 m × 4 mm i.d. glass column packed with Supelcoport (100/120 mesh) coated with 3 per cent. SP 2330.

Injector temperature : 250°C
Column temperature : 220°C (isothermal)
Detector temperature : 250°C
Carrier gas : nitrogen
Carrier gas flow-rate : 45 ml/min

If the erythritol internal standard is not isolated or quantified re-pack the first 5 cm of the GLC column and re-condition.

6. PROCEDURE

6.1 *Pre-treatment of Sample*

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

6.2 *Sampling*

Two identical sub-samples of the foodstuffs are taken and subjected to identical gelatinisation and enzymic treatments (see 6.5) followed by separate treatments. These separate treatments give values for

(a) The total NSP, including resistant starch but without distinguishing glucose derived from cellulose and that derived from non-cellulosic polysaccharides and resistant starch (see Section 6.6).
(b) The non-cellulosic polysaccharides including resistant starch (6.7). Using a further treatment (6.8) a separate value for RS is obtained.

6.3 *Analysis samples*

Accurately weigh, to the nearest 0·1 mg, between 200 and 500 mg of sample,

but containing not more than 200 mg of dry matter, into a 50-ml screw-top centrifuge tube (5.2) and add a stirrer.

Repeat with a second tube.

6.4 *Fat Extraction*

Dry samples (i.e. 90–100 per cent. of dry matter) with less than 2–3 per cent. of fat may be analysed directly. Otherwise add 40 ml of acetone (4.1), mix for 30 min using a magnetic stirrer, centrifuge and remove by aspiration as much of the supernatant as possible without disturbing the residue. Air-dry the residue or dry it by mixing at 62·5 ± 2·5°C (see 6.5.2).

6.5 *Dispersion and Enzymic Hydrolysis*

6.5.1 *Dispersion of the Starch*

Add 10 ml of sodium acetate buffer (4.3.2), add a teflon-coated magnetic stirring bar and then seal the tube tightly. Repeat with the second tube. Place the tubes in boiling water and leave for 2 h, timed from re-boiling, stirring continuously.

6.5.2 *Enzymic Hydrolysis of the Starch*

Cool each tube to 42 ± 2°C and immediately add 0·1 ml of mixed enzyme solution (4.6). (Note: for products with up to 100 mg of starch the enzyme solution may be diluted 1 : 2, and for products with only small amounts of starch 1 : 5.)

Incubate the samples at 42 ± 2°C for 16 to 18 h mixing continuously. After the enzyme treatment add 40 ml of absolute ethanol (4.2), mix well and leave to stand for 0·5 h at ambient temperature. Centrifuge at 2500 r.p.m. for 10 min or until a clear supernatant liquid is obtained. Remove by aspiration as much of the supernatant as possible without disturbing the residue and discard it.

Wash the residue twice with (2×) 50 ml of 80 per cent. ethanol (4.7) by mixing to form a suspension of the residue, centrifuging until clear and removing the supernatant as previously.

Add to the washed residue 40 ml acetone (4.1), stir for 5 min and then centrifuge at 1500 r.p.m., for approximately 10 min until clear. Remove the supernatant by aspiration and discard it.

Place the tubes in a water bath at 65·0 ± 2·5°C on the magnetic stirrer hot-plate and mix the residue for a few minutes until it appears to be dry. The beaker may be covered and the acetone vapour removed by water pump. Care must be taken to avoid loss of material at this stage.

6.6 *Determination of total NSP, including Resistant Starch*

6.6.1 *Acid Hydrolysis of the Residue from Dispersion and Enzymic Digestion (6.5)*

Disperse one of the dried residues from Section 6.5.2 in 2 ml of 12 mol/l sulphuric acid (4.8) using whirlimixer and the stirrer. Leave at 35 ± 2°C for 1 h to solubilise the cellulose and then rapidly add 12 ml of water followed by 10 ml of GLC internal standard solution (4.9). Mix the contents of the centrifuge tube.

Place in a boiling water bath for 2 h from re-boiling, stirring continuously. Cool to room temperature. Remove approximately half of the supernatant (for the determination of uronic acids—see 6.10). To the remainder add 1 drop of octan-2-ol (4.10), 3·0 g of barium carbonate (4.11) and stir for 30–40 min until neutral.

Centrifuge and take 2 ml of the supernatant for the determination of the neutral sugars by GLC (see Section 6.9).

6.7 *Determination of Non-Cellulosic Polysaccharides*

This procedure measures neutral non-cellulosic polysaccharide (NCP). The value for cellulose is the difference between glucose released in procedure A and procedure B. The gelatinisation, starch hydrolysis and washing procedures are identical to and run in parallel with procedure A.

6.7.1 *Acid hydrolysis of the dry residues after hydrolysis of starch (6.5.2)*

To the other dried residue from Section 6.5.2, add 10 ml of internal standard (4.9), 10 ml of 2 mol/l H_2SO_4 (4.17) and place in a boiling water-bath for 2 h mixing continuously. Cool to room temperature, remove (and discard) about 10 ml and to the remainder add a drop of octan-2-ol (4.10), 3 g of solid $BaCO_3$ (4.11) and stir until neutral. Centrifuge and take 2 ml of the supernatant for determination by GLC of the neutral sugars (see Section 6.9).

6.8 *Determination of Resistant Starch*

This modification of 6.7 allows a value for resistant starch, that is starch not hydrolysed by incubation with α-amylase and pullulanase, to be obtained in addition to information normally provided by procedure 6.7. The method is identical to procedure 6.7 until the treatment of the residue after starch hydrolysis.

6.8.1 *Hydrolysis of the dry residues after hydrolysis of the starch (6.5.2)*

To one of the dried residues from Section 6.5.2, add 2 ml of 2 mol/l KOH (4.12), mix for 0·5 h at room temperature with a whirlimixer and magnetic stirrer, add 10 ml of internal standard (4.9) and remove 0·6 ml while still mixing for the resistant starch measurement. To this 0·6 ml add 0·2 ml of 2 mol/l acetic acid (4.13), 0·5 ml amyloglucosidase solution (4.15) and incubate for 1 h at 65°C. Use the supernatant for GLC determination of glucose from resistant starch. To the remainder, add 2 ml 2 mol/l acetic acid (4.13), 13·4 ml, 2 mol/l H_2SO_4 (4.17) and heat for 2 h at 100°C. Cool to room temperature, add a drop of octan-2-ol (4.10), 3 g of solid $BaCo_3$ (4.11) and stir until neutral. Centrifuge, remove and mix 5 ml of supernatant with 2·5 ml of mixed resin (4.18). Allow to settle and take 2 ml of the supernatant for determination by GLC of the neutral NCP sugars (see Section 6.9).

6.9 *GLC Determination of Neutral Sugars*

6.9.1 *Preparation of Alditol Acetates*

6.9.1.1 *Pre-treatment of Sugar Solution*

To the supernatant obtained after Section 6.8.1 add 0·1 ml of 3 mol/l ammonium hydroxide (4.19).

6.9.1.2 *Reduction*

The procedures for reduction and acetylation of the sugar mixtures obtained from Sections 6.6.1, 6.7.1 and 6.8.1.1 are identical.

To 2 ml of neutral supernatant removed in these Sections, add 0·1 ml of ammonium hydroxide/sodium borohydride solution (4.20). Mix and leave for 2 h at ambient temperature. Add 0·5 ml of methanol (4.21), 0·1 ml of glacial acetic acid (4.22) and evaporate to dryness at 40°C.

Add a further 3 ml of methanol (4.21) to the residue and evaporate to dryness. Repeat this last step twice.

Treat 2-ml aliquots of the standard solutions (4.28) in the same way.

6.9.1.3 *Acetylation*

To the dry residues from 6.9.1.2 including the sugar standards, add 0·3 ml of acetic anhydride (4.23), put the screw-cap on, mix well and heat for 2 h at 120°C with frequent shaking during the first 10 min.

6.9.3 *GLC Determination*

6.9.3.1 Carry out conventional GLC determination of the prepared alditol acetate derivatives of the neutral sugar solutions obtained from Sections 6.6.1, 6.7.1 and 6.8.1.1 using the standards prepared from the standard sugar solutions in Section 4.28 and GLC columns and conditions given in Sections 5.6 and 5.7 and taking 1–2 μl of the supernatant solutions for injection.

6.10 *Uronic Acids*

Mix 0·3 ml of supernatant liquid obtained from Section 6.6.1 (diluted with water, if necessary, so that it contains between 20 and 80 μg/ml of uronic acids) with 0·3 ml of sodium chloride/boric acid mixture (4.24). Add 5 ml of concentrated sulphuric acid (4.25) and mix on a whirlimixer. Place the 50-ml tube in a heating block set at 70°C. Leave for 40 ± 1 min and then cool to room temperature by placing in water.

When cool, add 0·2 ml of dimethylphenol solution (4.26) and mix immediately. After 10–15 min read the absorbance at 400 and 450 nm in a solution spectrophotometer against a water reference cell. Subtract the reading at 400 nm from that at 450 nm for each sample. Plot the difference in absorbances obtained from glucuronic acid (4.27) standards (over the range 20–80 μg/ml) and read sample concentrations from the graph.

7. EXPRESSION OF RESULTS

7.1 *Calculation of Neutral Sugars*

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

$$\frac{\text{mg Internal Standard} \times \text{Response Sugar} \times 100}{\text{Response Internal Standard} \times \text{mg Sample}} = \text{per cent. sugar}$$

use myo-inositol as the internal standard.

7.2 *Total Non-Starch Polysaccharides including resistant starch (RS)—from Section 6.6*

Total NSP (including RS) = Arabinose + Xylose + Galactose + Glucose + Mannose + Uronic Acids + Rhamnose

Value includes resistant starch, cellulose and non-cellulosic polysaccharides.

7.3 *Cellulose—from Sections 6.6 and 6.7.1*

Cellulose = Glucose from 6.6—Glucose from 6.7.1

7.4 *Resistant Starch from Section 6.8.1*

Resistant starch = Glucose from 6.8.1

7.5 *Total Non-Cellulosic Polysaccharides*

Total NCP = Total NSP (including RS) from 7.2—cellulose from 7·3—RS from 7.4.

7.6 *Total Dietary Fibre*

Dietary fibre = Total NSP = Total NSP (including RS) from 7.2—RS from 7.4

or = Total NCP from 7.5 + cellulose from 7.3.

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

7.8 *Repeatability*

The difference between the results of two determinations of total dietary fibre, carried out simultaneously or in rapid succession on the same sample, by the same analyst, under the same conditions, shall not exceed [1·0] g per 100 g of sample.

8. NOTES

8.1 *Corrections*

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

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

8.2 Uronic acids may be expressed as glucuronic or galacturonic acid. The former predominates in cereal products, the latter in vegetable products.

Appendix III: "Standard" U.K. Methods for Dietary Fibre not Included in Trial

A. SOUTHGATE PROCEDURE

Southgate, following in the wake of McCance and Widdowson, was one of the first to appreciate the importance of dietary fibre in human nutrition[48,49].

Moreover, at an early stage he realised the inadequacies of available methods for measuring dietary fibre and, in particular, the pitfalls in translating methods devised for its measurement in animal nutrition to human foods. He therefore published, in 1969, a method for the measurement of dietary fibre[34], a method which with its subsequent modifications[35] has been the one most widely used in human nutrition. Southgate values for fibre in cereal foods and some vegetables and fruit are the basis of those in the U.K. Food Tables[36].

In this procedure, a fresh or air-dried sample is extracted with 85 per cent. of hot methanol, dried and ground. The sample is then extracted three times with boiling water. Water-soluble material (recovered by precipitation with ethanol) and water-insoluble material are then treated separately with takadiastase (or amyloglucosidase) to hydrolyse starch. The enzyme-treated residues (again precipitated with ethanol) are then hydrolysed with 5 per cent. of H_2SO_4 and constituent sugars measured as soluble and insoluble non-cellulosic polysaccharides by colorimetric methods. The residue after hydrolysis with 5 per cent. of H_2SO_4 is treated with 72 per cent. of H_2SO_4 and cellulose measured as the increase in glucose. Lignin is measured as "Residue − Ash".

This procedure has been criticised because of the incomplete removal of starch, the co-precipitation of carbohydrate with protein[43,50], for the use of enzyme preparations with hemicellulase activities[51], and for the use of non-specific colorimetric methods for the measurement of constituent sugars[52]. Table XIII compares values for the trial materials as measured by the Southgate method[35] or the Englyst procedure[24] as used in two of the collaborating laboratories. The difference for Sample D (cornflakes) is due to resistant starch but for white flour (Sample A) other factors must be responsible. For most of the trial samples the values by the two methods are similar.

B. METHOD OF SELVENDRAN AND DUPONT

In this procedure[37], an alcohol-insoluble residue (AIR) is prepared by homogenising the material with hot ethanol and then ball-milling for 15 h at 2°C in 90 per cent. ethanol. Starch in the AIR is gelatinised for 3 h at 85—90°C and then hydrolysed with α-amylase and pullulanase. The enzyme-treated product is dialysed, freeze-dried, and aliquots subjected to (1) 1 mol/l H_2SO_4 hydrolysis and (2) Saeman hydrolysis. Individual neutral sugars are measured by gas–liquid chromatography and uronic acids by the method of Blumenkrantz and Asboe-Hansen[38].

Table XI shows that the values obtained by this method are somewhat higher than those in the Englyst procedure although the recovery of constituent neutral sugars other than glucose is very similar in the two methods. Selvendran, when reporting his results for the trial samples, has suggested that the relatively higher glucose values for Samples A and B are due to a degraded starch–protein complex. However Samples A and B are unprocessed products (white flour and white flour + bran) and contain only negligible amounts of resistant starch. It seems unlikely therefore that the difference in glucose can be explained by a starch complex present in the samples. A more likely explanation is incomplete removal of starch, possibly because a starch complex is produced by the initial treatment used in the procedure. Detailed structural studies of wheat

endosperm cell walls[46,53] indicate that no more than 0·4–0·6 per cent. of dry matter in white flour is measured as NSP glucose. For cornflakes the difference is largely explained by a starch complex measured as resistant starch in the Englyst procedure but included as NSP by the Selvendran method.

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Book Review

STATISTICS FOR ANALYTICAL CHEMISTRY. J. C. Miller and J. N. Miller. Ellis Horwood Ltd., Chichester. 1984. Price Cloth, £18.50; Paperback, £7.50. 202 pp.

I have a book by E. L. Bauer called "*A Statistical Manual for Chemists*", published in 1971, and there was an earlier book by W. J. Youden entitled "*Statistics for Analytical Chemists*". Both were very good in their day. But as the authors of the book now under review state in their preface, one reason that has led them to write it is "the enormous impact of microelectronics, in the form of micro-computers and hand-held calculators, on statistics; these devices have brought difficult or lengthy statistical procedures within the reach of all practising scientists". This is very true, and it explains why most of the second half of this book discusses experimental designs for analytical research that, though very useful and productive, would not have been used twenty years ago, because the computations needed to produce maximum information therefrom either had not been worked out by statisticians or would have been impossibly lengthy and complex, even with the calculating machines available at that time. Now, the practicability of such computations means that a chemist who is seeking (for example) to increase the accuracy of an analytical method by varying its parameters is enabled to maximise the conditions with fewer determinations and more assurance than used to be possible.

While these are important considerations for the research worker, Public Analysts and heads of industrial control laboratories alike will be striving to obtain such information about each sample analysed as is relevant to the reason why it was submitted; they must therefore devote much attention to monitoring the analytical methods in use, to ensure that the output from the laboratory in terms of reports is as efficient as possible. "Efficiency" in this context means minimum cost per report while ensuring that each is as detailed, as accurate and as precise as is possible for its required purpose—but not more so if the unnecessary excess in any direction has cost unnecessary money.

For such top people—and for all their subordinates whose ambitions range beyond the laboratory bench—a basic knowledge of statistics is in these days essential, and to this end the first part of this book should be very useful. While the primary principles are expounded as simply as the subject itself permits, many illustrative examples are drawn from analytical experiments and data, and each chapter ends with a number of "test" problems of the same sort, with the solutions at the end of the book.

The important question of the treatment of "outliers" in a set of replicate observations is well discussed in this book (though it is rather surprising that

Cochran's test is not mentioned as well as Dixon's) from a strictly statistical point of view. But the very practical point is not made that the *reason* why the outlier occurred should be looked for, particularly if the set of observations in which it was found can be compared with other similar sets. This is very relevant to a matter with which all Public Analysts have become increasingly concerned lately—collaborative assays, where in the general case w replicates of an analysis by a prescribed method are performed by each of x analysts on each of y samples in each of z laboratories, so that when complete there are available $wxyz$ results for comparison. When properly designed and the results efficiently computed, such assays can yield as much important information about the laboratories as about the method, and it is the responsibility of the Head of each laboratory to go into this aspect of the results as completely as possible.

If (for example) the results from one laboratory are found to include three outliers when there are only three in those from all the other participating laboratories together, there is a strong suggestion that all is not well with the analytical skill of that laboratory as applied to that method, even though the mean result is very close to the other means. Again, a laboratory may obtain a set of replicates agreeing very closely with each other but the mean may differ significantly from those of the other laboratories, pointing to a systematic error arising within the laboratory that is out of step—and which the Head may never have suspected had he not been able to use the other laboratories' results for comparison. The subject of computing the results of collaborative assays is hardly dealt with at all in the book under review; the matter is mentioned on pp. 151/2 as an example of the use of an analysis of variance, but in a book intended for practical chemists the exposition could usefully have been considerably extended.

The latter part of the book will be of interest mainly to research workers trying to improve the precision of an analytical method or the yield in a manufacturing process, and it is noteworthy that to a large extent the book describes what can be done and the principles involved but assumes that anyone really interested will have access to a computer and to a programme suitable to his needs. I agree that having regard to modern conditions, this is completely acceptable.

I have no hesitation in recommending this book to all who wish to become familiar with the principles on which the science of statistics is based and even to be able to make the simpler computations for themselves; and perhaps even more importantly, to be able to understand not so much how an expert statistician conducts his computations as the practical meaning of the conclusions he reaches. I am sure that any M.Chem.A. could, by using this book, acquire at least that degree of expertise in applied statistics; I am equally sure that no Head of a laboratory can be efficient in its management without such knowledge. My advice to any such person who doubts his own abilities in this direction is—get this book.

E. C. Wood