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A Rapid Method for Determining the Carbohydrate Component of Dietary Fibre

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ABSTRACT

A simplified procedure for the determination of the non-starch polysaccharides in foods is described. Starch is removed by treatment with heat-stable α-amylase and resistant starch by treating with dimethyl sulphoxide (DMSO) and amyloglucosidase. Total neutral sugars and uronic acids are then measured colorimetrically in the acid hydrolysates of the starch-free material.

The method is more rapid than the complex fractionation procedures (Southgate, 1969; Selvendran et al., *1979; Englyst, 1981) but does not provide a detailed composition. The time per sample compares favourably with the gravimetric assay of Asp* et al. *(1983) and a direct measure of the carbohydrate content of the dietary fibre fraction is obtained. The results compare favourably with those obtained by the complex method of Englyst* et al. *(1982). The method is applicable to a range of foods, both raw and cooked, and should have application in the routine analysis of dietary fibre since it is comparatively rapid and does not require complex or expensive equipment.*

INTRODUCTION

Ever since the term 'dietary fibre' (DF) came into wider use (Trowell, 1972), problems of definition have arisen (Spiller *et al.*, 1976; Trowell,

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1976) and no real consensus has yet emerged as to which dietary components (see review by Spiller *et al.*, 1978) should be included in a definition of DF; this makes DF determination a difficult task for the analyst.

Currently, for research purposes, those methods that give the greatest detail (Southgate, 1976; Englyst *et al.*, 1982; Selvendran & DuPont, 1980) have been employed, since it is possible to compile a total DF to suit a given definition by the summation of various components. These methods, however, are time-consuming, difficult for untrained staff and provide more information than is necessary. They are unsuitable for quality control where a robust, reproducible and rapid method is needed to produce a single total value for defined components.

In order to fulfill these objectives we have examined rapid destarching methods and the measurement of the residual non-starch polysaccharides (NSP) by various colorimetric methods on the solubilised and hydrolysed NSP. The data have been compared with that obtained by gas–liquid chromatography of the hydrolysed NSP and the method of Englyst *et al.* (1982).

MATERIALS AND METHODS

Materials

Analytical grade reagents from BDH or Sigma were used, α-amylase (Termamyl 120L) was a gift from Novo Industri A/S, Copenhagen, and amyloglucosidase from *Aspergillus niger* (E.C.3.2.1.3) was obtained from Boehringer, Cat. No. 102857.

Methods

Sample preparation

High moisture samples (tomato pulp, runner bean pods, potato) were freeze dried, low moisture samples (cereals, flour) were used as received and high fat (>5%w/w) samples (hazelnuts) were defatted by extracting 5 g in 3 × 25 ml boiling 80% v/v ethanol followed by 3 × 25 ml boiling acetone. All samples were finely ground (<1·0 mm) and thoroughly mixed prior to use. Bread samples (14, as 7 blind duplicates),

as used in the second Ministry of Agriculture, Fisheries and Food dietary fibre methods trial, were used as provided.

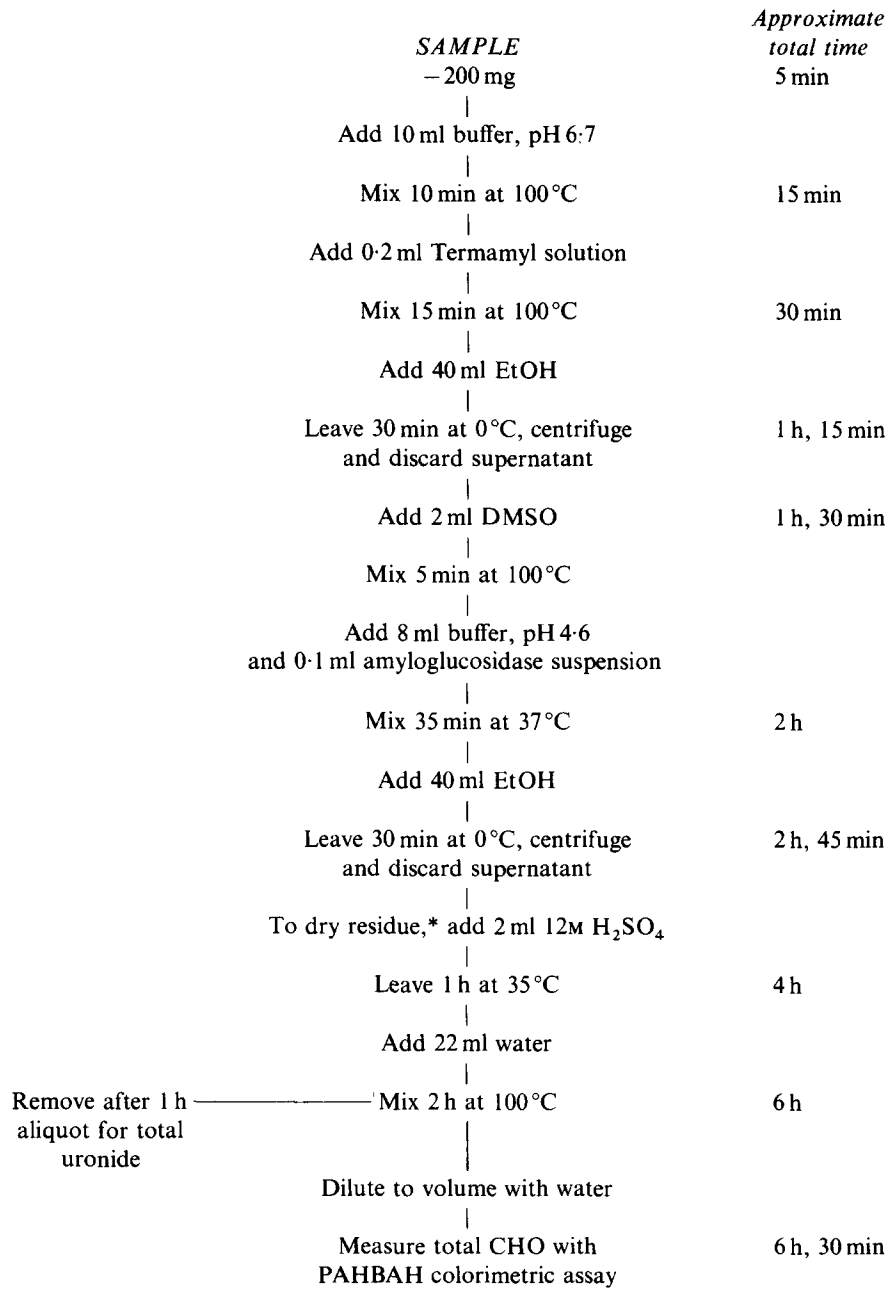
Analysis

A flow diagram with approximate times is given in Fig. 1. Approximately 200 mg of sample was accurately weighed into 50-ml glass screw top centrifuge tubes and 10 ml 0·1M Tris-maleate buffer, pH 6·7 (Gomori, 1953) in $2{\cdot}5 \times 10^{-3}$M $CaCl_2$, was added, together with a 12 mm magnetic stirrer bar. The solid was thoroughly dispersed and the tubes incubated at 100 °C in a rapidly boiling water bath (a large beaker on a stirrer-hotplate) with continuous stirring to gelatinise the starch.

After 10 min, 0·2 ml Termamyl 120L (previously diluted 25× with water) was added and the sample incubated at 100 °C for a further 15 min with continuous stirring. After incubation the tubes were removed from the water bath, 40 ml of ethanol were added and the tubes were then placed in an ice-water bath for 30 min to precipitate any solubilised DF components. The precipitate was pelleted by centrifugation at 2000 *g* for 15 min at ambient temperature and the supernatant discarded by aspiration. The pellet was dispersed in 2 ml of dimethyl sulphoxide (DMSO) and the tubes replaced in the boiling water bath for 5 min with continuous stirring. The tubes were removed, 8 ml of 0·1M acetate buffer, pH 4·6 (Gomori, 1953), and 0·1 ml of amylglucosidase suspension were added, thoroughly mixed and incubated at 37 °C for 35 min. The residue after digestion was recovered by ethanol precipitation and centrifugation as before, then oven-dried at 100 °C.

After cooling to ambient temperature, 2 ml of 12M H_2SO_4 were added, the dry pellet thoroughly dispersed using the stirrer bar and incubated at 35 °C for 1 h. To the dispersed sample, 22 ml of water were added and the samples replaced in the boiling water bath for 45 min (partial hydrolysis) or 2 h (total hydrolysis) with continuous stirring. After hydrolysis the samples were made up to 100 ml with water. The carbohydrate content of the partially hydrolysed samples was measured against glucose standards using the 'total' carbohydrate methods of Roe (1955) and Mejbaum (1939) as modified by Southgate (1969), Devor (1950) and Dubois *et al.* (1956).

The neutral sugar content of the fully hydrolysed samples was determined directly, using the automated procedure of Hudson *et al.* (1976) or by GLC as alditol acetates (Englyst *et al.*, 1982) and uronic acids by the method of Scott (1979). Glucose was used as the standard for the



* Dry residue may be kept for analysis next day.

Fig. 1. Scheme for the rapid estimation of dietary fibre.

colorimetric method, GLC response factors were found for each neutral sugar and inositol was used as an internal standard. Uronic acids were measured using a galacturonic acid standard. NSP were also measured by the method of Englyst *et al.* (1982).

All the results were expressed as monosaccharides or uronic acid monomer on a dry matter basis.

RESULTS AND DISCUSSION

In any measurement of non-starch polysaccharides (NSP), the complete removal of starch is essential. This is not as simple as might first appear since the starch must be gelatinised if it is to be readily hydrolysed enzymically. Most native starches will gel at 100 °C but some legume starches may need to be autoclaved (121 °C). On cooling starch gels, however, retrogradation occurs with the formation of amylose crystallites (small aggregates of highly structured hydrogen-bonded amylose) (Collinson, 1968) and other complexes, e.g. starch-lipid (Holm *et al.*, 1983). Some of these structures are not thermo-reversible and will therefore be resistant to enzymic hydrolysis. Most cooked starchy foods will therefore contain a proportion of resistant starch (Englyst *et al.*, 1983). In order to render resistant starch susceptible to enzymic hydrolysis, chemical treatment with alkali (Englyst *et al.*, 1982) or DMSO is normally used. Since, in a method suitable for quality control, speed is essential, a dual approach was adopted using heat stable α-amylase (Termamyl) to remove the bulk of the starch, followed by a chemical treatment (DMSO at 100 °C) to disrupt the resistant starch prior to a secondary enzymic treatment.

Termamyl 120L is a mixture of enzymes, only the α-amylase being heat resistant. Addition of this enzyme mixture is therefore carried out at 100 °C to avoid any hydrolytic losses that might be caused by other carbohydrate-degrading enzymes. Furthermore, with high starch products, for example, white flour, starch hydrolysis stops short of 100 %, no further hydrolysis occurring after 15 min under the conditions used here. It would appear that the α-amylase is inhibited by its own products since a second treatment with Termamyl 120L of the 80 % ethanol-precipitated destarched residue results in further starch removal.

Tris-maleate buffer (Gomori, 1953) with added calcium was chosen for the α-amylase digestion; the avoidance of citrate and phosphate was felt

TABLE 1

Glucose Found by GLC After Acid Hydrolysis of Destarched Food Samples (grams per 100 grams dry matter)
(Cellulose[a] glucose in parentheses)

Enzyme	*Cornflakes*	*Soya flour*	*White flour*	*Runner beans*	*Potato*	*Tomato*	*Hazelnuts*	*Wheat bran*	*Rolled oats*
Termamyl 120L	12·8	1·7	12·3	12·9	14·9	6·6	2·2	13·9	12·8
Termamyl 120L + Amyloglucosidase	3·0	0·6	0·8	11·0	2·2	7·7	1·5	9·8	4·5
Amylase + Pullulanase[b]	3·8 (0·2)	2·1 (1·7)	1·6 (0·4)	16·1 (15·4)	2·5 (2·0)	7·8 (7·4)	2·2 (1·9)	11·3 (8·2)	4·9 (0·6)

[a] NSP cellulose, Englyst *et al.* (1982).

[b] Enzyme mixture used by Englyst *et al.* (1982).

to be desirable as free Ca^{2+} ions are required to maintain the stability of the enzyme at elevated temperature. The efficiency of starch removal, as reflected in the glucose content of acid hydrolysed residues of foods after starch removal, is shown in Table 1.

It can be seen that Termamyl 120L is least efficient at hydrolysing starch in those samples where there is a large amount of starch or that have been cooked (e.g. cornflakes), more efficient in those containing raw starch and most efficient where there are only trace amounts of raw starch (e.g. tomato, runner bean).

The combination of Termamyl 120L and amyloglucosidase is, in all cases, marginally more effective in removing starch than the α-amylase + pullulanase mixture used by Englyst *et al.* (1982).

It has been shown (Selvendran *et al.*, 1979) that the neutral sugar monomers most commonly found in DF hydrolysates are stable in 1M H_2SO_4 at 100 °C for 2 h. These conditions were therefore chosen for the dilute acid hydrolysis of the DF polysaccharides.

Rhamnogalacturonan is, however, difficult to hydrolyse quantitatively in 1M H_2SO_4 and only about 60% of the rhamnose is recovered (Selvendran & DuPont, 1980). Uronic acid content is therefore most easily determined using a concentrated acid colorimetric method on the partially hydrolysed DF after 1 h at 100 °C.

The optimum conditions of Saeman prehydrolysis (Saeman *et al.*, 1954) of plant cell wall material are not well defined. The effects of strong (12M or 72%) sulphuric acid on carbohydrates, particularly the pentoses, can be severe (Rasper *et al.*, 1981) if the conditions are not strictly controlled. Dispersal of the dry DF fraction in 12M H_2SO_4 for 1 h at 35 °C was found to give the greatest yield of monomers after dilution to 1M and hydrolysis for 2 h at 100 °C (Table 2). These are the same conditions as those employed by Englyst *et al.* (1982).

Carbohydrate determination

Methods for carbohydrate assay are broadly divided into two types; (a) 'total' methods using concentrated acid (Dische, 1962) and (b) methods using the reducing capacity of the sugars (Hodge & Hofreiter, 1962). The potential advantage of the 'total' methods is that the reactions occur equally readily with polysaccharides and monomers so there is no need to hydrolyse the polysaccharide fraction of DF prior to determination. However, the methods of Roe (1955) and Mejbaum (1939), modified by

TABLE 2
Effect of Time in 12M H_2SO_4 at 35°C on the Yield of Neutral Sugars and Uronide from Dry Runner Bean Pods (grams per 100 grams)

Time (min)	*Uronide*[a]	*Total neutral sugars*[b]	*Total*
15	6·73	21·5	28·3
30	7·38	20·6	27·9
45	7·14	23·9	31·0
60	7·44	23·9	31·4
90	7·75	21·2	28·9
120	8·00	21·6	29·6

[a] Method of Scott (1979).
[b] By GLC.

Albaum & Umbreit (1947) and later by Southgate (1969), Devor (1950) and Dubois *et al.* (1956) do not give similar results at a given concentration, and produce different absorption maxima for hexoses, pentoses and uronic acids. The methods are also prone to non-specific interference. The application of these methods to the partially hydrolysed carbohydrates, from the range of foods examined, failed to give useful correlations with the NSP data derived from the procedure of Englyst *et al.* (1982). However, these 'total' methods may have application to foods where the amount, but not the composition, of DF is variable or where the composition of the DF is known and mixed standards used.

Methods for total reducing sugars rely on the reduction of a suitable chromogen to a coloured product, usually in alkaline solution. Most widely used are the reduction of Cu^{2+}, $Fe(CN_6)^{3-}$,2,4,6-trinitrophenol (Picric acid) and 2-hydroxy 3–5 dinitrobenzoic acid (dinitrosalicyclic acid, DNSA). The reduction reactions are seldom stoichiometric (Davidson, 1967), the reaction conditions are critical (Miller, 1959) and the rates of reaction with the different sugars are different (Miller *et al.*, 1961). Furthermore, these methods exhibit variable responses to different sugar monomers and reproducibility is often poor, even in automated Technicon systems (because of reagent sensitivity to atmospheric oxygen). The reducing and 'total' methods are therefore unsuitable for the measurement of the complex mixtures of neutral sugars and uronic acids present in non-starch polysaccharides.

More recently, Lever (1972) investigated the use of acid hydrazides as colorimetric reagents for reducing sugars. The reaction involves

condensation of 3-hydroxy benzoic acid hydrazide (PAHBAH) with the reducing sugar in alkaline solution to produce an intensely coloured anion. The method has been successfully automated to a Technicon system (Hudson *et al.*, 1976), is sensitive to less than 1 μg/ml of glucose and gives broadly similar response to all the reducing sugars and uronic acids normally found in hydrolysed DF fractions.

This method also has the added advantage that there is little interference from other components that may be present in DF hydrolysates (e.g. protein). It therefore offers a viable alternative to the more usual methods of carbohydrate determination.

Table 3 shows the response of PAHBAH to a range of carbohydrates.

TABLE 3
Relative Response of PAHBAH to Some Carbohydrates

Carbohydrate	*Relative response (Glucose = 100)*
Glucose	100
Galactose	64
Mannose	88
Xylose	80
Arabinose	78
Rhamnose	82
Galacturonic acid	40
Polygalacturonic acid (PGUA)	1
Hydrolysed PGUA[a]	4
Sucrose	4

[a] PGUA hydrolysed in 1M H_2SO_4 for 2 h at 100 °C.

The response relative to glucose is, in all cases, lower. The Table shows that where one monomer is present in very large amounts the results obtained should be significant underestimations. This underestimation is greatest for uronic acids and it is clearly necessary to develop an approach to correct for this. Galactose alone would also produce an underestimation (30 %) of the sugar present. However, most DF from natural sources is a mixture in which glucose (from cellulose and β-glucans) and pentose sugars predominate and, in practice, one would expect the underestimation to be more modest than the values for the monosaccharides would suggest. Mixtures of glucose, arabinose and galacturonic acid gave the predicted response, and a three-dimensional

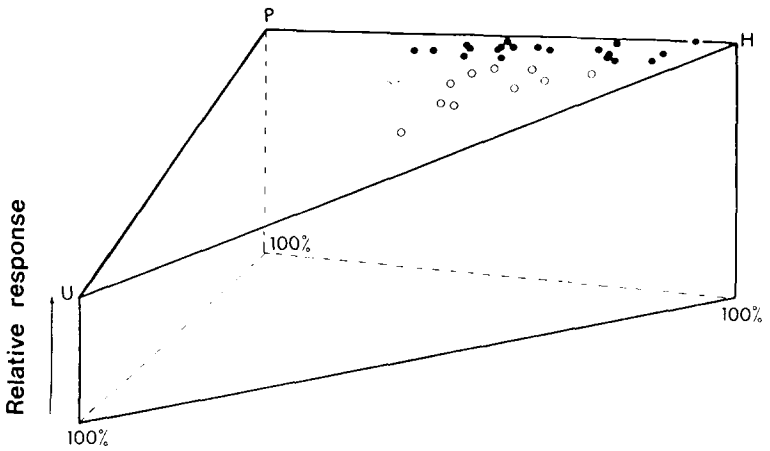


Fig. 2. Response of PAHBAH to mixtures of hexose, pentose and uronic acid. Points represent a range of NSP from some monocot and dicot food plants. ●, Cereals; ○, Dicotyledons; P, Pentose (arabinose); H, Hexose (glucose); U, Uronide (galacturonic acid).

Figure (Fig. 2) gives a plane on which the response to all possible mixtures may be found. The Figure also shows the theoretical responses of NSP hydrolysates of known composition (Englyst *et al.*, 1982).

The monocotyledons and dicotyledons occupy distinct areas of the plane. It can be seen that the cereals contain very little uronide (*ca.* 3%) and are composed mainly of hexoses and pentoses. The ratio of hexose:pentose ranges from 50:50 to 90:10, giving responses of 87% to 97% of the glucose standard. However, the dicotyledonous DF hydrolysates contain more uronide and are, therefore, subject to greater underestimation, particularly since the recovery of uronide as the monomer after hydrolysis is poor.

Since the plane is sloped in two dimensions, each point has a unique value. It should therefore be possible to obtain DF compositional data—and hence detect adulteration from a single determination of a known weight of DF.

Cereals (monocotyledons) contain little uronide (usually glucuronic acid) but the fruit and vegetables (dicotyledons) may contain up to 10% (galacturonic acid). Because of the low response (40%) for galacturonic acid and the low yield of uronic acid monomers on acid hydrolysis, their contribution to the colour yield with PAHBAH will be very small (Table 3). Although this may not be of importance in cereal DF, fruit and

vegetable DF may be underestimated by as much as 30%. This underestimation can, however, be readily corrected by measuring the uronide content in the acid hydrolysate after 1 h by the method of Scott (1979).

The DF values of the range of foods and bread samples by the method of Englyst *et al.* (1982), the proposed colorimetric method and GLC of the acid hydrolysates are given in Table 4.

Despite the fact that the theoretical response of PAHBAH to sugar mixtures should result in an underestimate of the DF content if read against glucose standards, in almost every case PAHBAH gave greater values than the GLC measurement of the neutral sugars. In those samples of high uronide content, if corrections are applied for uronide, then PAHBAH gives consistently high values. The methods, however, are highly correlated. PAHBAH + uronide − V − GLC + uronide, $r = 0{\cdot}9935$, slope = 0·9375, intercept = − 0·2648.

This consistent overestimation could be caused by synergistic effects of the sugar mixtures on the colour response or the presence of positively interfering materials. Synergistic effects were reported by Hudson *et al.* (1976) but were not found in mixtures of neutral sugars.

However, it is possible that some loss of sugars occurs during acid hydrolysis and that the degradation products react with PAHBAH to give a positive response at 410 nm. The most likely acid degradation products are furfural, hydroxymethyl furfural and laevulinic acid. These three compounds give 3%, 1·5% and 0·2% of glucose response, respectively. No synergistic effects were observed with glucose or xylose in the presence of 10% of the compounds. The reasons for overestimation are still unresolved. Using a t test to decide whether the regression line intercept differs from zero and the slope from one, there are significant differences between our Englyst data and that from the PAHBAH + uronide. Where y = Englyst data and x = PAHBAH + uronide, the intercept (T_α) and the slope (T_β) are both highly significantly (***) different. The 95% confidence limits for the intercept (α) are −1·82, −0·72 and, for the slope (β), 1·08, 1·17. Although these limits span a small range, it is apparent that, in common with most chemical analyses, the greatest proportional differences occur in those samples which have a low DF value. Within the range of values in the study the differences between the methods are less than one unit of DF as derived from the confidence limits of the regression.

Comparison of the colorimetric procedure and the method of Englyst

TABLE 4
Comparison of DF Values Obtained by Different Methods
(Grams per 100 grams dry matter)

Sample	*PAHBAH*[a]	*GLC*[b]	*Uronide*[c]	*GLC+ Uronide*	*PAHBAH+ Uronide*	*Englyst*[d]
Cornflakes	3·72	3·24	0·12	3·37	3·84	1·11
Hazelnuts	4·01	3·91	1·96	5·87	5·97	6·87
Runner beans	25·4	21·6	9·59	31·2	35·0	37·9
Potato	5·45	4·45	1·28	5·73	6·73	6·69
Tomato	12·2	11·7	7·95	19·7	20·1	21·6
Wheat bran	31·2	29·8	1·67	31·5	32·9	36·3
Oats	8·18	6·54	0·38	6·92	8·56	7·54
Bread 1	3·05	2·26	0·01[i]	2·27	3·06	2·54
2	9·29	7·54	0·29[f]	7·83	9·58	8·94
3	5·16	4·87	0·13[k]	5·00	5·29	5·13
4	6·55	5·59	0·18[g]	5·77	6·73	5·89
5	6·74	6·48	0·69[e]	7·17	7·43	5·31
6	6·42	5·61	0·19[g]	5·80	6·61	7·05
7	2·95	2·86	0·02[i]	2·88	2·97	2·46
8	3·62	3·62	0·02[j]	3·64	3·64	2·94
9	4·86	4·71	0·10[h]	4·81	4·96	4·41
10	8·03	8·09	0·28[f]	8·37	8·31	8·78
11	6·79	5·96	0·70[e]	6·66	7·49	6·77
12	3·58	3·37	0·02[j]	3·39	3·60	2·99
13	4·57	2·71	0·09[h]	2·80	4·66	4·12
14	5·04	4·13	0·12[k]	4·25	5·16	5·33

[a] Proposed colorimetric method.
[b] Neutral sugars in acid hydrolysate.
[c] Uronide in acid hydrolysate by method of Scott (1979).
[d] Englyst *et al.* (1982), not including resistant starch.
[e] High fibre white bread containing pea hull flour.
[f] Wholemeal bread.
[g] High fibre brown bread.
[h] Mixture of *g* and *i*.
[i] White bread.
[j] Rye bread.
[k] Low fibre brown bread.

TABLE 5
Reproducibility of the Rapid Procedure and GLC Determination of Neutral Sugars + Uronide. Results %DM Basis.

Runner bean pod		*Cornflakes*	
PAHBAH + Uronide	*GLC + Uronide*	*PAHBAH + Uronide*	*GLC + Uronide*
30·0	29·1	3·95	4·00
31·2	29·7	3·80	3·81
31·4	32·4	4·06	4·14
32·3	32·4	3·88	3·79
32·5	29·9	3·88	3·98
$\bar{x}$ 31·5	$\bar{x}$ 30·7	$\bar{x}$ 3·91	$\bar{x}$ 3·94
SD 1·0	SD 1·6	SD 0·10	SD 0·15

et al. (1982) indicates that this rapid method gives values that, although statistically significantly different, correlate well with the complex method, PAHBAH + uronide – V – Englyst, $r = 0{\cdot}9900$, slope = 1·1983, intercept = – 0·9052. The repeatability of the rapid method and that for the GLC estimation of the neutral sugars + uronide are given in Table 5.

CONCLUSIONS

A consistent and reliable value for TDF may be obtained by this method for both cooked and raw cereals. However, the presence of pectin in fruit and vegetables means that an additional assay must be made for uronide. Some fruit and vegetables that contain only trace amounts of starch may not need a secondary treatment with DMSO and amyloglucosidase. Enhanced accuracy may be achieved by the use of suitable mixed standards where the composition of the DF hydrolysate is known.

The method has not been tested on the hydrocolloid gums but it is unlikely to give useful results for those materials high in uronic acids (e.g. alginate and gum arabic) or which contain sulphated residues (e.g. carrageenan).

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