Proceedings of the Nutrition Society (1996), **55**, 863–880

PROCEEDINGS OF THE NUTRITION SOCIETY
A meeting of the Scottish Section of the Nutrition Society was held at the University of Dundee on 2/3 April 1996

Symposium on 'The nutritional consequences of complex carbohydrates'

Symposium 1
The analysis of complex carbohydrates: relevance of values obtained *in vitro*

BY M. M.-J. CHAMP
Laboratoire de Technologie Appliquée à la Nutrition, INRA, rue de la Géraudière, BP 1627, 44316 Nantes cedex 03, France

Since the 1970s, complex carbohydrates have been considered as an important component in various dietary guidelines, especially in the US. However, the term has been used in this same country with various definitions (Table 1). Thus, the consumers are aware of the recommendations to increase their consumption of complex carbohydrates, but they are not clear what the term represents, and are unable to find this information readily on the food label. It seems to be accepted by most scientists that it should include starches, and NSP or dietary fibre. However, the use of 'complex carbohydrates' in food labelling is not widespread. This might be due to several reasons, including: (1) this new term will probably be confusing for the consumer who is familiar with other terms; (2) the word 'complex' also means 'intricate', which is not a suitable description for a substance to be mentioned on a food label. However, the use of 'dietary fibre' to define the carbohydrates which are not available in the small intestine seems to be widespread, even if a few countries, including the UK, prefer the term 'NSP'.

In 1988, the British Nutrition Foundation decided to convene an independent Task Force, to review the state-of-the-art of nutritional and medical aspects of dietary fibre. Complex carbohydrates, defined as starches plus NSP, rather than 'fibre', were chosen for various reasons (British Nutrition Foundation, 1990) including: (1) the recommendations to increase 'fibre-rich' carbohydrates, (2) the properties of resistant starch (RS), which is fermentable and exhibits similar properties to those of the NSP (Englyst *et al.* 1987*c*). Complex carbohydrates were defined in the British Nutrition Foundation (1990) report as all carbohydrate polymers which contain twenty or more monosaccharide residues. This definition is thus close to that of polysaccharides for which the limit for the number of residues is often established at twenty. As a consequence, the simple carbohydrates will include the mono-, di-, tri-, and tetra-saccharides and sugar alcohols present in foods as well as other oligosaccharides containing up to nineteen residues. It has to be admitted that this limit is purely conventional and does not have any physiological basis. Moreover, the alcoholic solution (800 ml/l) that is often used to precipitate the polysaccharides is not specific, since many fructans, glucofructans and arabinans are soluble in such solutions.

According to Association of Official Analytical Chemists (AOAC) International, complex carbohydrates should include, for labelling purposes, both dietary fibre and the digestible complex carbohydrate fraction of food (Lineback *et al.* 1995). This was also the

Table 1. *Brief history of the term 'complex carbohydrates' in the USA and UK*

Year	Document and author(s)	Recommendations and/or definition
USA		
1977	*Dietary Goals for the United States* US Congress, Senate	Increased consumption of CC Digestible carbohydrates or starch
1980	*Nutrition and Your Health: Dietary Guidelines for Americans* US Department of Agriculture and US Department of Health and Human Services	Increased consumption of foods containing CC
1988	*The Surgeon General's Report on Nutrition and Health* US Department of Health and Human Services	Increased consumption of CC and fibre (diets with whole-grain cereal products, vegetables and fruits)
1989	*Recommended Dietary Allowances and Diet and Health: Implications for Reducing Chronic Disease Risk* National Research Council	Increased CC consumption CC = digestible CC and dietary fibre
1991	US Nutrition Facts/Nutrition Labels (cited by Lineback *et al.* 1995)	CC = digestible polysaccharides DP >9
1992	*Food Guide Pyramid* US Department of Agriculture and US Department of Health and Human Services	Increase consumption of CC relative to other foods
1993	US Regulation	The term 'complex carbohydrates' is not allowed on the nutrition label
1995	*Definition and Analysis of Complex Carbohydrates/Dietary Fiber* Booklet of abstracts AOAC International Workshop	
1996	*AOAC/AACC Collaborative Study of Complex Carbohydrates Analysis Methods* (S. S. Lee, Kellogg's)	CC = available starch + dietary fibre
UK		
1990	*Complex Carbohydrates in Foods* British Nutrition Foundation	A polysaccharide containing twenty or more monosaccharide residues
1994	*Starchy Foods in the Diet* British Nutrition Foundation	CC = starch + NSP

CC, complex carbohydrates; DP, degree of polymerization; AOAC, Association of Official Analytical Chemists; AACC, American Association of Cereal Chemists.

conclusion of an international survey on complex carbohydrates initiated by the Kellogg Company and the US Food and Drug Administration (Lee & Prosky, 1996).

Several classifications of complex carbohydrates can then be proposed. None of them is entirely satisfactory in that the boundaries between each of the categories are not absolute, either structurally or functionally, nor are the boundaries susceptible to precise analytical demarcation. Although the majority of the complex carbohydrates in the human diet are derived from foods of plant origin, a range of polysaccharides is present in animal tissues and these also contribute to complex carbohydrate intake.

GENERAL CONSIDERATIONS ON ANALYSIS OF COMPLEX CARBOHYDRATES

Until recently, there was no method which could be used to measure all the complex carbohydrates in a single analysis. However, an on-going interlaboratory study is aiming to evaluate a new method derived from dietary fibre analysis as outlined in the AOAC methods (Lee *et al.* 1996). It includes an HPLC step to quantify 'available' starch.

The first difficulty in setting up such a method is that all the starch, both available starch and RS, has to be analysed properly. The separate analysis of NSP and starch (RS + available starch) meets this requirement whereas a separate analysis of total dietary fibre (AOAC method, Prosky *et al.* 1988) and total starch would overestimate available starch content by measuring part of the RS in the dietary fibre residue. The new method proposed by Lee & Prosky (1996) should solve this problem as 'available' starch is analysed within a modified AOAC dietary fibre method (Lee *et al.* 1996). The sample is subjected to sequential enzymic digestion using heat-stable α-amylase (*EC* 3.2.1.1), protease and amyloglucosidase (*EC* 3.2.1.3), precipitated with 4 vol. ethanol, and filtered. The filtrate is analysed for mono- and disaccharides, using HPLC and two enzymic methods.

The second problem is linked to the definition of the oligosaccharides or polysaccharides to be considered as dietary fibre or NSP. Depending on the definitions, the criteria are:
a (lower) limit in the degree of polymerization (DP ≥ 10, 12 or 20; chemical definition),
unavailability in the small intestine (physiological definition),
insolubility in alcoholic solution (800 ml/l or 80° Gay-Lussac or ethanol–water (4:1, v/v); analytical definition).
The first criterion necessitates a chromatographic method to evaluate quantitatively the amount and size of polymers of DP 10–20, depending on the definition. The second would require a human nutrition study (with ileostomates or intubated healthy volunteers) or an analytical method which would have been validated with *in vivo* studies with the types of oligosaccharides which are considered in the sample. Finally, the third criterion is simple but does not have any physiological significance, because of the various solubilities of carbohydrate polymers in alcoholic solutions.

Thus, for labelling purposes, the principle underlying the new complex carbohydrate analysis or the separate analysis of NSP and starch could be adopted. However, a consensus should be obtained on a precise definition. For scientific purposes, the separation of the various polysaccharides present, with specific analysis of individual neutral and acidic sugars, might be necessary (Fig. 1). The main methods used for analysing starch and dietary fibre or NSP as well as the main undigestible oligosaccharides will be presented and discussed.

MEASUREMENT OF TOTAL STARCH

Starch has long been analysed using a polarimetric method. This method is still used in the animal-feed industry because it allows a quick analysis of the starchy material of the feed at the different steps of the process. However, this method has been largely abandoned in favour of precise analysis of starch in food and feed, because of numerous artefacts (amino acids, oligosaccharides, simple sugars etc.).

Any enzymic analysis of starch implies four major steps:
preparation of the samples. To allow a proper analysis of starch, it has to be fully accessible to amylolytic enzymes. Grinding, therefore, should be efficient enough to disrupt the cell

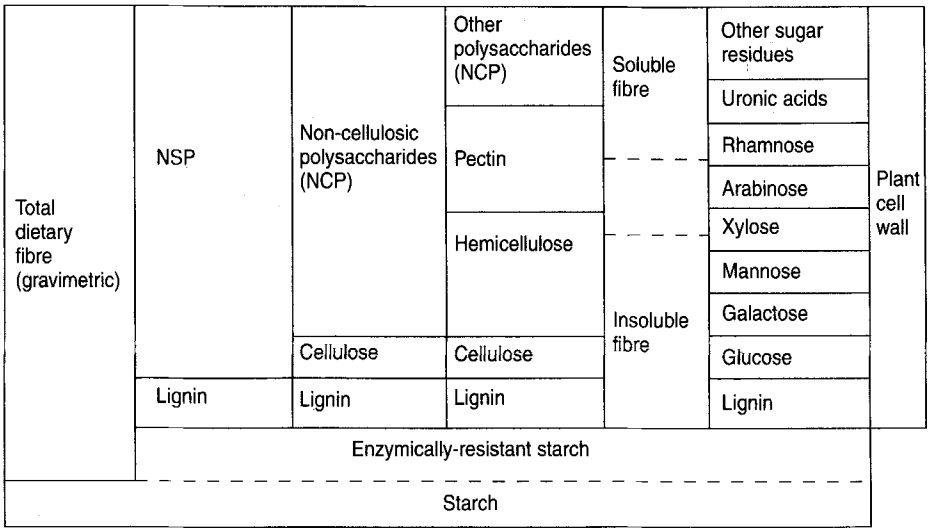

Fig. 1. Relationships between the different methods of measuring dietary fibre. (– – –), Boundaries that are not absolute. (From British Nutrition Foundation, 1990.)

walls and/or the protein network surrounding the starch granules;
dispersion of the starch. In order to be completely hydrolysed by the amyloglucosidase, the starch must be decrystallized and amylose and amylopectin must be accessible to the enzyme. This step was originally performed using boiling water and autoclaving (Thivend *et al.* 1965). The use of dimethylsulphoxide (DMSO) was then proposed (Holz, 1977; Cuber, 1982), but now most methods suggest the use of alkali such as NaOH and KOH (2 M final concentration in the dispersion medium; Karkalas, 1985; Englyst *et al.* 1992);
hydrolysis of starch. Starch is hydrolysed to glucose by an amyloglucosidase. The enzyme is an endoenzyme (from *Aspergillus niger*) able to hydrolyse both α-(1–4) and α-(1–6) linkages;
quantification of glucose. If properly ground, dispersed, then hydrolysed, starch should be totally analysed as glucose. The choice of the analytical method for glucose should be guided by the expected concentration of glucose as well as by the possible presence of pigment(s) in the sample. The complex hexokinase (*EC* 2.7.1.1)–glucose-6-phosphate dehydrogenase (*EC* 1.1.1.49; HK–G6PDH) is very sensitive and does not have any contra-indication, whereas the use of the glucose oxidase (*EC* 1.1.3.4)–peroxidase (*EC* 1.11.1.7) complex (GOD–POD) should be avoided in the presence of pigments such as biliary pigments (for example, digestive contents).
GOD–POD or glucose oxidase–1-phenyl-2,3-dimethyl-4-aminopyrazolone-(5) (GOD–PAP): GOD–POD

$$\text{D-glucose} + H_2O + O_2 \xrightarrow{\text{GOD}} H_2O_2 + \text{gluconic acid},$$

$$H_2O_2 + DH_2 \xrightarrow{\text{POD}} 2H_2O + D,$$

where DH_2 is, for example, 4-aminoantipyrine + 4-hydroxybenzoic acid (Merckotest, Merck, Darmstadt, Germany) and D is N-(4-antipyryl)-*p*-benzoquinone imine.

https://doi.org/10.1079/PNS19960085 Published online by Cambridge University Press

HK–G6PDH

$$\text{D-glucose} + \text{ATP} \xrightarrow{\text{HK}} \text{glucose-6-phosphate},$$

$$\text{glucose-6-phosphate} + \text{NADP}^+ \xrightarrow{\text{G6PDH}} \text{gluconate-6-phosphate} + \text{NADPH} + \text{H}^+.$$

NADPH absorbance is proportional to the amount of glucose present in the sample after the complete hydrolysis of starch.

MEASUREMENT OF DIETARY FIBRE AND NSP

Many reviews have compared the various methods available for the analysis of dietary fibre. These methods are usually classified into three categories:
gravimetric methods;
colorimetric methods;
chromatographic methods which allow the determination of monomeric composition of polysaccharides (GLC and HPLC).

Gravimetric procedures for dietary fibres

Gravimetric methods measure an insoluble residue by weighing, after chemical or enzymic solubilization of the digestible constituents (proteins, starch and fats). Some methods are also available to quantify soluble dietary fibre components after precipitation with alcohol, or after ultrafiltration or dialysis.

The crude fibre method (Horwitz, 1980), or Weende method, was developed in the 1850s for determining indigestible material in feed and forages. It should not be used for human or most animal (especially single-stomached animal) food. The method is simple and involves sequential extraction with dilute acid (12·5 ml H_2SO_4/l) and alkali (12·5 ml NaOH/l), and isolation of the insoluble residue by filtration.

Among the detergent methods (neutral-detergent and acid-detergent fibre), those developed by Van Soest and collaborators (Van Soest, 1963; Van Soest & Wine, 1967; Robertson & Van Soest, 1977, 1981) have certainly been the most widely used. Since the publication of the first version, several adaptations have been proposed by this group and others to allow the analysis of dietary fibre in most foods, whereas the method was initially devised for analysis of feeds and forages. The main disadvantage of this method is that soluble components are not determined and cannot easily be recovered from the filtrate. The first step includes extraction with a 'neutral detergent' (sodium dodecyl sulphate, EDTA and amylase) of most digestible components (including protein, fat and starch) but also pectins. The second step allows the solubilization of most hemicellulose with cetyl-methylammonium bromide in H_2SO_4 solution. In the third step, cellulose is solubilized in H_2SO_4 (720 ml/l). Finally, lignin is defined as the weight loss on ashing of the residue. The relative amounts of the main components of dietary fibre (hemicellulose, cellulose and lignin) are calculated from weights of the residues after each step.

Several enzymic–gravimetric methods for analysis of indigestible residues have been proposed since the last century. Most of these methods are described in a number of reviews (Asp & Johansson, 1984; Schweizer, 1984, 1986). The method which is the most extensively used throughout the world is the gravimetric procedure developed

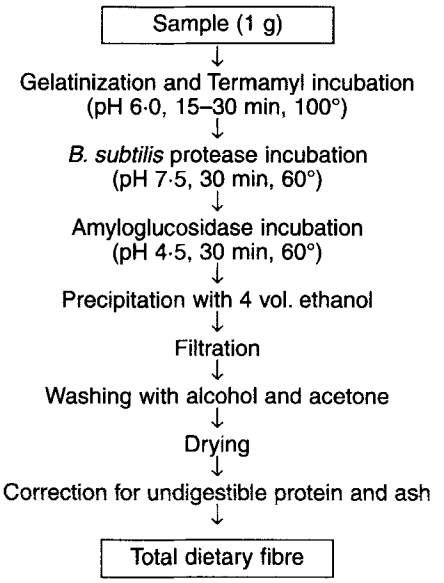

Fig. 2. Analytical scheme for total dietary fibre: the Association of Official Analytical Chemists method of Prosky *et al.* (1985, 1988). Amyloglucosidase, *EC* 3.2.1.3; *B. subtilis*, *Bacillus subtilis*. (From Asp & Johansson, 1984; Schweizer, 1989.)

collaboratively by the Association of Official Analytical Chemists (Prosky *et al.* 1984, 1988). It measures the residue after enzymic removal of protein and starch; the residue weight is corrected for protein and ash (Fig. 2). This analysis provides a value for total 'dietary fibre' which includes most NSP (soluble and insoluble), lignin, and part of the RS.

Measurement of total NSP

These methods aim to purify cell-wall material which can then be analysed. Soluble fibre is recovered from the enzymic liquors by dialysis. The main features of a procedure for measurement of NSP are summarized in Fig. 3. The principal stages are as follows:

1. extraction of free sugars and lipids using aqueous alcohols and organic solvents;
2. removal of starch, which includes a dispersion step (DMSO) then an enzymic hydrolysis of starch (glucoamylase or α-amylase + pullulanase (*EC* 3.2.1.41));
3. recovery of NSP after ethanolic precipitation (800 ml ethanol/l) by filtration or centrifugation. In some cases, dialysis and freeze-drying may be preferable.
4. hydrolysis of NSP using 12 M-H_2SO_4 at ambient (or below) temperature followed by dilution to 1 or 2 M and heating at 100° for 2·5 h (Saeman hydrolysis). In order to specifically quantify cellulose, non-cellulosic polysaccharides (NCP) are hydrolysed by dilute 1 M- or 2 M-H_2SO_4 for 2·5 h at 100°, the difference between total NSP and NCP providing the value for cellulose.
5. measurement of neutral sugars and uronic acids. Total sugars can be measured colorimetrically. Individual neutral sugars can be measured after separation by HPLC. GLC analysis is an alternative but requires derivatization in alditol acetates (reduction to the alditols and acetylation). Uronic acids have to be analysed separately, usually using colorimetric methods (Fig. 4).

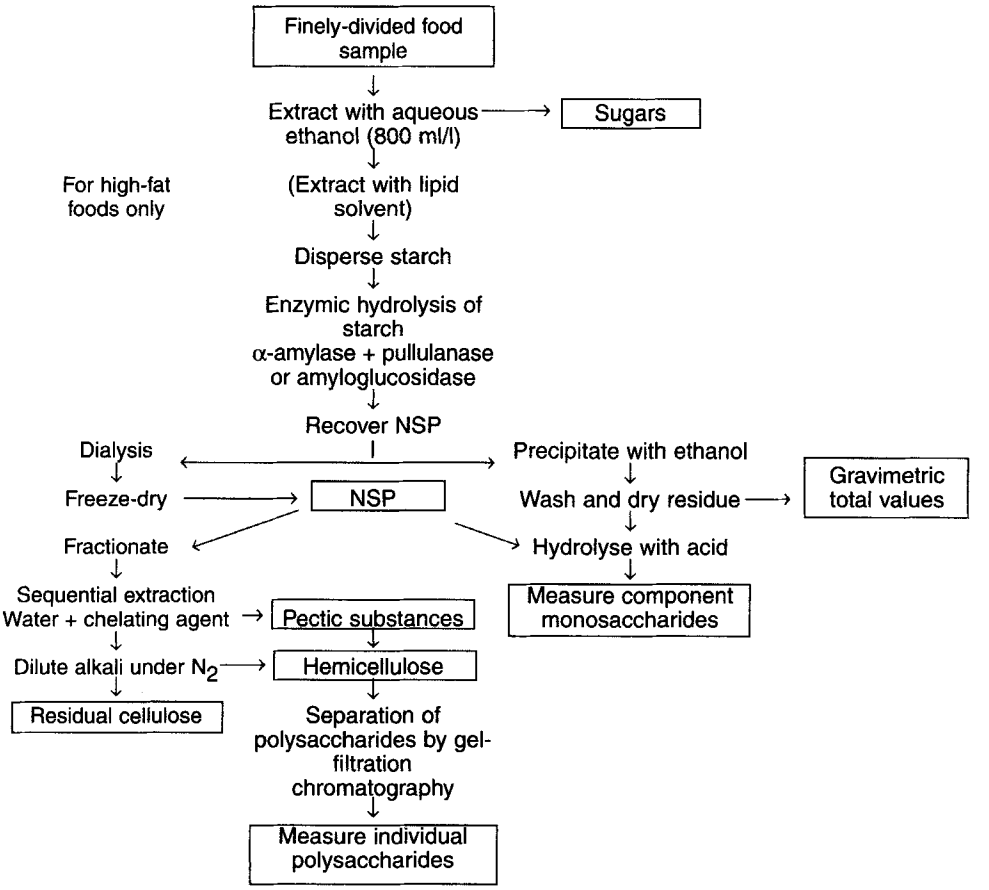

Fig. 3. Main features of the measurement of total dietary fibres or fractions. α-Amylase, *EC* 3.2.1.1; pullulanase, *EC* 3.2.1.41; amyloglucosidase, *EC* 3.2.1.3. (From British Nutrition Foundation, 1990.)

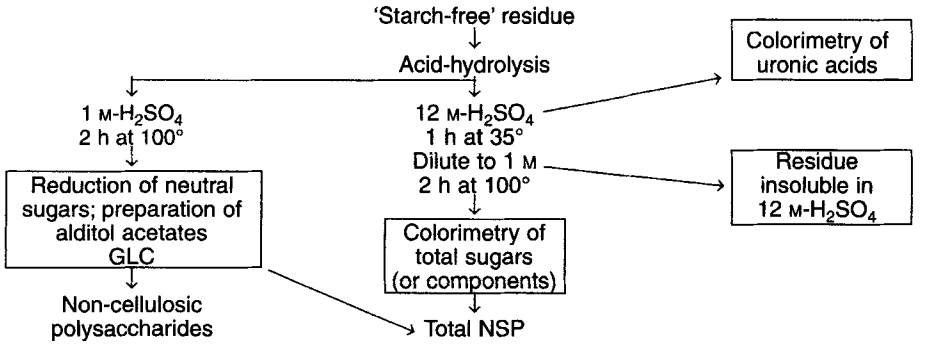

Fig. 4. Direct measurement of polysaccharides in NSP. (From British Nutrition Foundation, 1990.)

The Englyst enzymic method (Englyst *et al.* 1982) is the main method available for the measurement of NSP, quantified as their component monosaccharides and uronic acids after hydrolysis. The values exclude lignin and RS (Englyst & Cummings, 1987), thus,

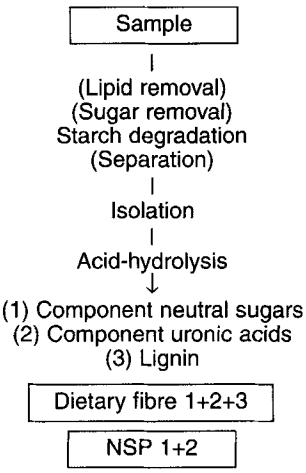

Fig. 5. General scheme for dietary fibre analysis with chemical methods. (From Schweizer, 1989.)

NSP values are substantially less than values obtained by the gravimetric methods, especially for heat-treated starchy products.

Chemical methods for dietary fibre analysis

In strong acid solution, carbohydrates undergo condensation reactions with a large number of substances giving coloured products which can be measured spectrophotometrically. Three such reactions (those with anthrone, orcinol or carbazole) are, under certain conditions, relatively specific for hexoses, pentoses and uronic acids respectively. The mutual interference between groups of sugars is most pronounced with the orcinol method; therefore, corrections of interferences have to be performed. Moreover, when complex samples are analysed, starch and/or oligosaccharides may have to be deducted if the aim of the analysis is to determine dietary fibre.

Southgate (1969) was probably the first to propose a chemical method to quantify unavailable carbohydrates. Presently the best-known chemical methods are the GLC method of Theander & Westerlund (1986) and of Englyst & Cummings (1988). In GLC methods, the monomeric constituents of dietary fibre polysaccharides are liberated by acid-hydrolysis, usually derivatized to their corresponding alditol acetates and separated by GLC. Uronic acids need a specific determination by colorimetry or by decarboxylation. HPLC has been used more recently for the determination of dietary fibre monomers (Slavin & Marlett, 1983).

The general scheme for dietary fibre analysis by chemical methods is presented in Fig. 5, whereas their main characteristics are summarized in Table 2. Comparative analyses have been performed between these methods showing that the Theander & Westerlund (1986) method gives very similar results to the enzymic–gravimetric method of Asp (Siljeström *et al.* 1986).

MEASUREMENT OF RESISTANT STARCH

RS has been defined as 'the sum of starch and products of starch degradation not absorbed

Table 2. *Comparison of main characteristics of four chemical methods for total dietary fibre as NSP + lignin or as NSP (neutral sugars + uronic acids)* (From Schweizer, 1989)

Authors	Starch removal	Acid-hydrolysis	Measurement of fibre components: Neutral sugars	Uronic acids	Lignin
Theander & Westerlund (1986)	(1) Termamyl (0·5 h, 96°) (2) Amyloglucosidase (16 h, 60°)	(1) 12 M-H_2SO_4 (1 h, 30°) (2) 0·4 M-H_2SO_4 (6 h, 100° or 1 h, 125°)	GC*	Decarboxylation†	Gravimetry
Englyst & Cummings (1988)	(1) DMSO (1 h, 100°) (2) α-Amylase + pullulanase (16 h 42°)		GC*	Colorimetry‡	Not analysed
Faulks & Timms (1985)	(1) Termamyl (15 min, 100°) (2) DMSO (5 min, 100°) (3) Amyloglucosidase (35 min, 37°)	(1) 12 M-H_2SO_4 (1 h, 35°) (2) 1 M-H_2SO_4 (2 h, 100°)	Colorimetry§	Colorimetry‡	Not analysed
Englyst & Hudson (1987)	(1) DMSO (1 h, 100°) (2) α-Amylase + pullulanase (16 h, 42°)		Colorimetry‖	Colorimetry‖	Not analysed

DMSO, dimethylsulphoxide; amyloglucosidase, *EC* 3.2.1.3; α-amylase, *EC* 3.2.1.1; pullulanase, *EC* 3.2.1.41.
* As alditol acetates.
† Before acid-hydrolysis.
‡ With 3,5-dimethylphenol.
§ With *p*-hydroxy benzoic acid hydrazide.
‖ With 3,5-dinitrosalicylic.

https://doi.org/10.1079/PNS19960085 Published online by Cambridge University Press

in the small intestine of healthy individuals' (Asp, 1992). It should be analysed, therefore, using a method which mimics the digestion of starchy food in the upper part of the digestive tract. Several methods have been published during the past 15 years, some of them being validated on the basis of *in vivo* measurements.

Increasing knowledge of the process of starch digestion in human subjects has allowed a new classification of RS which is widely approved (Englyst *et al.* 1992; Table 3). Three classes of RS have been identified:

physically-inaccessible starch (RS1). It is found in partly-milled grains and seeds. Legumes such as beans or lentils are known to be one of the main sources of RS. The preparation and cooking process is of great importance in the RS content of the food;

RS granules (RS2). B-type starch when uncooked is known to be very resistant to enzymic hydrolysis (B-type refers to the X-ray diffraction pattern of the starch). Raw starches have been classified into three main types: A (most cereal starches, cassava starch), B (potato and banana starches) and C (most legume starches). Banana is the main source of RS2 in the human diet and although starch is converted into simple sugars and sucrose during ripening of the fruit, partially-ripe banana still has a significant amount of starch. Most of the cooking procedures are able to gelatinize raw starches allowing the disappearance of RS2 in the food;

retrograded starch (RS3). It is present in most starchy foods which have been cooked, then cooled and stored from several hours to several months. The retrogradation is a recrystallization of starch molecules which occurs after gelatinization and which involves mostly the linear fraction of the starch, the amylose. However, amylopectin can also retrograde over a much longer term than amylose. Potatoes cooked then cooled have been shown to contain RS (Englyst & Cummings, 1987). Reheating of starch reduces the RS content of the potato showing that the retrogradation is partly reversible. Several cycles of heating then cooling allow an increase in RS.

RS1, RS2 and RS3 can coexist in a same food. Indeed a meal of beans contains both RS1 and RS3 (Noah *et al.* 1995) whereas RS1 and RS2 are both present in bananas (Faisant *et al.* 1995*a*).

During the past 5 years, a lot of effort has been put into the evaluation, then into the improvement of the existing methods for analysis of RS. Several analytical methods are now proposed in the literature.

General principle

In order to quantify RS, the first step is to remove the digestible starch from the sample. This is performed using a pancreatic α-amylase. In some of the methods, an amyloglucosidase is added in order to avoid a possible inhibition of the α-amylase by the products of the digestion (mainly maltose, maltotriose). The amylolysis is also sometimes preceded by a proteolysis which is supposed to reflect the action of the pepsin (*EC* 3.4.23.1) inside the stomach and of the trypsin (*EC* 3.4.21.4) which is secreted in the pancreatic juice together with the α-amylase.

After the hydrolysis of the digestible starch, RS is quantified directly in the residue (isolated most of the time by ethanol (800 ml/l) precipitation; Champ, 1992; Faisant *et al.* 1995*b*) or by difference between total starch and digestible starch, which are quantified separately (Englyst *et al.* 1992).

Table 3. In vitro *nutritional classification of starch* (From Englyst *et al.* 1992)

Type of starch	Example of occurrence	Probable digestion in small intestine
Rapidly-digestible starch	Freshly-cooked starch food	Rapid
Slowly-digestible starch	Most raw cereals	Slow but complete
Resistant starch		
1. Physically-inaccessible starch	Partly-milled grains and seeds	Resistant
2. Resistant starch granules	Raw potato and banana	Resistant
3. Retrograded starch	Cooled cooked potato, bread, and cornflakes	Resistant

Main analytical methods

The method of Björck *et al.* (1986) quantifies the starch residue present in the dietary-fibre residue obtained by the methods of Asp *et al.* (1983) or Prosky *et al.* (1988). The procedure is described in Fig. 6(a). Its main characteristics are that (1) some of the samples such as bread can be analysed as eaten without further drying, (2) the samples are submitted to a triple hydrolysis by Termamyl (100°, 15 min), pepsin and pancreatin. The main consequence of this procedure is the gelatinization of all raw starch present in the sample which cannot be quantified as RS. The main advantage of this method is that it can be performed with total-dietary-fibre analysis. However, it quantifies mostly retrograded starch and it has been validated using a rat model (antibiotic-treated rat) (Björck & Asp, 1991).

The method proposed by Englyst *et al.* (1992) is strongly supported by British scientists. The various types of RS are determined by controlled enzymic hydrolysis and measurement of the released glucose using glucose oxidase. RS is defined as the starch not hydrolysed after 120 min incubation with pancreatic amylase and amyloglucosidase at 37°. It is calculated by deducting from total starch content, the slowly-digestible starch and the rapidly-digestible starch contents of the sample hydrolysed respectively after 120 and 20 min incubation (Fig. 6(b)). The main characteristics of this procedure are that:

1. fresh foods can be analysed as eaten. They are passed through a mincer. The hydrolysis is performed in presence of glass balls and guar gum (to prevent mechanical alteration of very sensitive starch such as raw potato starch);
2. the samples are hydrolysed with pancreatin, amyloglucosidase and invertase (*EC* 3.2.1.26). The pancreatin contains several enzymic activities including α-amylase and proteolytic enzymes which allow digestion of the digestible material (starch and proteins). On the other hand, the lipolytic activity of the pancreatin might be ineffective due to the absence of bile in the medium. The invertase is added in order to hydrolyse all the sucrose present in the sample into glucose and fructose. The sucrose must then be quantified separately. This step has been introduced into the method because of the presence of an invertase-contaminating activity in the amyloglucosidase. This activity could result in an overestimation of the starch content of the sample;
3. the digestible starches (starches hydrolysed after 20 and 120 min hydrolysis) are quantified using an ethanolic extract (644 ml ethanol/l). The extract is hydrolysed into glucose by amyloglucosidase. Then the glucose is quantified using GOD–PAP kit;

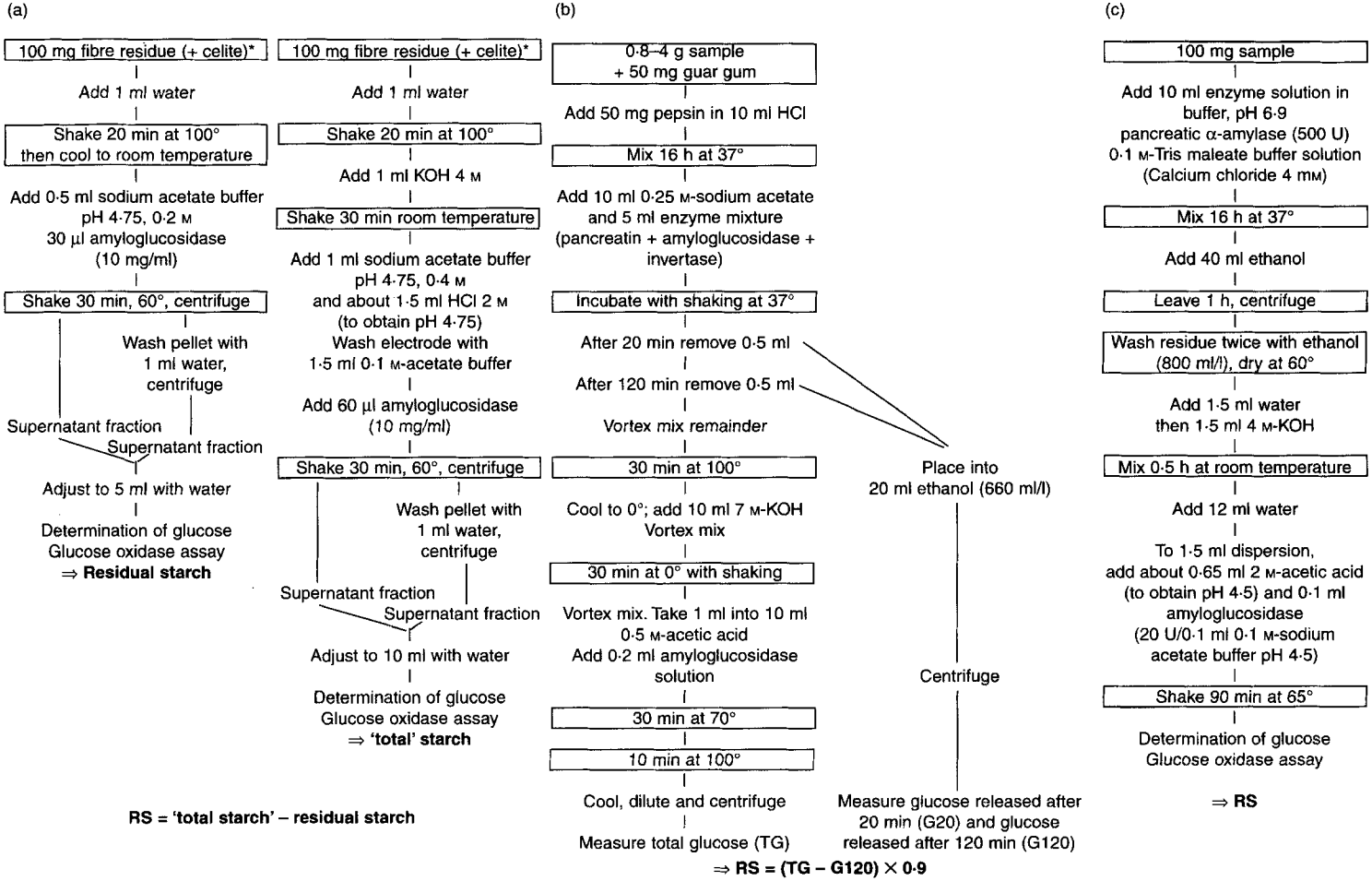

Fig. 6. Main analytical methods for the quantification of resistant starch (RS). (a) Method of Björck *et al.* (1986) quantifying starch residue present in the dietary-fibre residue. *The fibre residue–celite mixture isolated by the method of Asp *et al.* (1983) or Association of Official Analytical Chemists (Prosky *et al.* 1988). (b) Method proposed by Englyst *et al.* (1992). (c) Method described by Berry (1986) and modified by European FLAIR-Concerted Action on Resistant Starch participants (Champ, 1992). Amyloglucosidase, *EC* 3.2.1.3; α-amylase, *EC* 3.2.1.1; invertase, *EC* 3.2.1.26; glucose oxidase, *EC* 1.1.3.4; pepsin, *EC* 3.4.23.1.

https://doi.org/10.1079/PNS19960085 Published online by Cambridge University Press

4. RS content is determined by difference between total starch and digestible starch contents.

Undoubtedly, this method has been optimized (especially conditions of hydrolysis) to be able to quantify most types of RS. It has been validated with a number of *in vivo* studies performed on ileostomates. However, the method is usually considered as long, and poor reproducibilities are observed without a long period of training. Moreover, it requires very specific equipment (mincer, shaking water-baths) and enzymes which are not all commercially available.

Although most of the development of analytical methods for RS has been performed in Europe, an Australian group has been developing its own method (Muir & O'Dea, 1992, 1993). It is probably the most physiological method due to the initial preparation of the sample (standardized chewing of the sample), but has many similarities to the method developed by Englyst *et al.* (1992). This method has also been validated with *in vivo* studies on ileostomates (Muir & O'Dea, 1993; Muir *et al.* 1995). From the seven comparisons available, it seems satisfactory. No comparison with other *in vitro* methods has been published. Since it has been used only by the authors until now, difficulties and reproducibility cannot be evaluated.

After the interlaboratory study within the European FLAIR – Concerted Action on Resistant Starch (EURESTA) programme, we adapted the method described by Berry (1986) and slightly modified by the EURESTA participants (Champ, 1992) (Fig. 6 (c)) to fit with available *in vivo* values (A. M. Langkilde and H. Andersson, unpublished results; Faisant *et al.* 1995*b*; Champ *et al.* 1996). As in most methods, *in vitro* RS is defined as the starch not hydrolysed by incubation with α-amylase. Hydrolysis products are extracted in ethanol (800 ml/l) and discarded. RS is then solubilized with 2 M-KOH and hydrolysed with amyloglucosidase. In the new procedure (Champ *et al.* 1996), amyloglucosidase is added to the pancreatic α-amylase to avoid inhibition of the amylase by the products of the digestion. In the first two methods (Champ, 1992; Faisant *et al.* 1995*b*), the sample has to be dried (freeze-drying when possible) and ground into fine particles. In the new procedure, fresh samples can be analysed after grinding in a mincer which is supposed to simulate chewing of the food. This step is derived from the procedure adopted by Englyst *et al.* (1992). Unlike most methods the amount of test material which is used in the test is derived from its total starch content. NaN_3 is now used to prevent bacterial proliferation (and degradation) during the overnight amylolysis. In the Champ (1992) method the ethanolic precipitate containing RS was washed then dried with acetone. The drying has now been omitted (Faisant *et al.* 1995*b*; Champ *et al.* 1996). Finally, RS is then dispersed using KOH before the amyloglucosidase hydrolysis into glucose. A first step of gelatinization has been added in the last two methods to facilitate starch dispersion. The advantages of this method are that it is simple and relatively rapid. Ten samples can easily be analysed (in duplicates) in a normal day of work and no particular training is needed. The first methods (Champ, 1992; Faisant *et al.* 1995*b*) were optimized using values provided by Englyst *et al.* (1992). The new procedure (Champ *et al.* 1996) has recently been validated in collaboration with Langkilde and Andersson using *in vivo* values obtained from ileostomates.

Comparison of the main methods

Few comparisons have been performed using exactly the same samples. However, one has

Table 4. *Comparison of resistant starch (RS; g/kg total starch) data determined* in vivo *and* in vitro

Source of starch	*in vitro* RS: Englyst *et al.* (1992)	*in vitro* RS: Faisant *et al.* (1995*b*)	*in vitro* RS: Champ *et al.* (1996)	*in vivo* RS
				Ileostomy
Potato starch, raw	665	830	777	788*
HACS: Raw	714	722	528	503*
Retrograded	305	364	296	301*
Bean flakes	106	124	112	90–109†
Corn flakes	39	49	43	31–50‡
				Intubation
Beans, canned	171	–	171	165§

HACS, high-amylose maize starch.
* A. M. Langkilde and H. Andersson, personal communication.
† Schweizer *et al.* (1990).
‡ Englyst *et al.* (1992), Muir & O'Dea (1992).
§ Noah *et al.* (1995).

been published by Champ (1992) and Englyst *et al.* (1992) within the EURESTA programme. As expected, the method of Björck *et al.* (1986) provides the lowest values. However, significant underestimation is observed only for native and treated pure starches. The Englyst *et al.* (1992) and Champ (1992) methods give very similar values. The method modified by Faisant *et al.* (1995*b*) provides a significantly higher RS value for raw potato starch; this value is closer to the *in vivo* value obtained with ileostomates (Langkilde & Andersson, 1994). The comparison of the new method (Champ *et al.* 1996) with the Englyst *et al.* (1992) method has been performed on a sample of fresh and mashed beans. Both methods provided exactly the same results: 73 g RS/kg DM.

Some of the *in vitro* methods have been validated using *in vivo* data which are in most cases obtained with ileostomates. *In vitro* data from Champ *et al.* (1996) appeared to be satisfactory when compared with *in vivo* data obtained on ileostomates (data from Langkilde & Andersson, 1994) with the same starchy foods (Table 4).

RESISTANT OLIGOSACCHARIDES

Several resistant oligosaccharides can be found naturally in foods (inulin, α-galactosides) or are commercially available (Table 5). Some are considered to have beneficial effects on health; they are called 'bifidus factors' because they seem to increase bifidobacteria. They also bind to the specific glycoconjugate receptors present on the surface of the epithelial cells which play an important role in the attachment of pathogens and their toxins. Thus, there is a competitive inhibition of this attachment by specific oligomers. They are known to ferment in the large intestine like most 'conventional' dietary fibre. These products are not properly quantified by AOAC (Prosky *et al.* 1988) and Englyst *et al.* (1992) methods in which soluble fibre is precipitated in aqueous ethanol (780–800 ml/l). Therefore, specific methods have to be performed to quantify these oligosaccharides. The most commonly

Table 5. *Some commercially-available oligosaccharides resistant to digestion* (From Schols *et al.* 1995)

Products	Production methods
β-Fructo-oligosaccharides	(a) Transfructosylation of sucrose
	(b) Hydrolysis of inulin
α-Galacto-oligosaccharides	Isolation from soyabean whey
β-Galacto-oligosaccharides	Transgalactosylation of lactose
Lactulose	Isomerization of lactose
Isomalto-oligosaccharides	Transglucosylation of liquefied starch
Xylo-oligosaccharides	Enzymic hydrolysis of xylans
Chito-oligosaccharides	Enzymic hydrolysis of chitin

used method so far is HPLC using an amino-bonded silica column with acetonitrile and water mixtures as eluent (Dysseler *et al.* 1994). GLC can also be used but involves a derivatization of the samples. The most promising method is probably high-performance anion-exchange chromatography in which detection is usually performed using a pulsed electrochemical detector in the pulsed amperometric detection mode. Thus, Quemener *et al.* (1994) proposed a specific method of analysis of inulin and oligofructose. It involves enzymic hydrolysis (amyloglucosidase + inulinase and amyloglucosidase alone) whereas NaOH (0·16 mol/l) was used as an eluent. β-Galacto-oligosaccharide mixtures have been analysed using mixtures of water – 0·1 M-NaOH then 1 M-sodium acetate in 0·1 M-NaOH as eluents (Schols, 1995). However, the elution behaviour might be difficult to interpret when complex oligosaccharides mixtures are analysed.

On the other hand, the AOAC dietary fibre method has been adapted to allow analysis of products containing inulin (Quemener *et al.* 1994) or polydextrose (Arrigoni, 1995).

LIGNIN

Some definitions of dietary fibre include lignin. Complex carbohydrates being often defined as the sum of available starch and dietary fibre, lignin should be included in the 'complex carbohydrates'. However, lignins are not chemically carbohydrates, they are macromolecules in which phenylpropane monomers are mainly linked by aryl-alkyl-ether chemical bonds (Monties, 1995). A complete review has recently been published on that complex class of components (Monties, 1995); therefore, no detailed procedures will be presented here. The monomeric compositions of the lignins can be determined by chromatographic methods after acidolysis and thioacidolysis.

Lignins, being insoluble in water and closely associated with the cell-wall matrix can be easily quantified by spectroscopic and gravimetric determinations. Thus, they can be estimated by u.v. spectroscopy after solubilization. The gravimetric determinations of lignins remain the reference procedure even if systematic occurrence of solvolysis has been reported. This phenomenon induces the formation of both insoluble, as 'repolymerized', and soluble, as 'solvated', products.

CONCLUSIONS

According to Slavin (Lineback *et al.* 1995), the classification of complex carbohydrates should not specify the minimum chain length of saccharides. Dietary recommendations insist on the fact that consumers should increase their intake of a variety of foods rich in complex carbohydrates and preferably of 'intact foods' such as fruits and vegetables. There is growing acceptance that isolated carbohydrates provide different physiological responses from those produced by whole foods. However, the recent interest in non-available carbohydrates having prebiotic activities will probably extend the definition of dietary fibre and consequently of complex carbohydrates. A recent questionnaire relating to complex carbohydrates showed that most scientists agree that they should include, for labelling purposes, both dietary fibre and the digestible polysaccharide fraction of food. Only 30% of the respondents checked lignin and resistant oligosaccharides as complex carbohydrates. However, in the previous two international surveys on dietary fibre definition and analysis, respondents generally supported the view that the dietary fibre definition should include lignin and resistant oligosaccharides in addition to NSP and RS (Lee & Prosky, 1996). The answers to the different international surveys reveal an obvious contradiction which shows that the scientific community is still uncertain of the definition and the use of both terms, 'dietary fibre' and 'complex carbohydrates'.

REFERENCES

Arrigoni, E. (1995). Determination of polydextrose: an approach. In *Proceedings of a Workshop Physico-chemical Properties of Dietary Fibre and Effect of Processing on Micronutrients Availability*, pp. 69–74 [A. Sorensen, K. E. Bach Knudsen, E. Gudmand-Hoyer and M. Nyman, editors]. Luxembourg: Office for Official Publications of the European Communities.

Asp, N.-G. (1992). Preface: Resistant starch – Proceedings from the Second Plenary Meeting of EURESTA:European FLAIR Concerted Action No. 11 on physiological implications of the consumption of resistant starch in man. *European Journal of Clinical Nutrition* **46,** Suppl. 2, S1.

Asp, N. G. & Johansson, C.-G. (1984). Dietary fibre analysis. *Nutrition Abstracts and Reviews in Clinical Nutrition* **54**A, 735–752.

Asp, N. G., Johansson, C.-G., Hallmer, H. & Siljeström, M. (1983). Rapid enzymatic assay of insoluble and soluble dietary fiber. *Journal of Agricultural and Food Chemistry* **31,** 476–482.

Berry, C. S. (1986). Resistant starch: formation and measurement of starch that survives exhaustive digestion with amylolytic enzymes during the determination of dietary fibre. *Journal of Cereal Sciences* **4,** 301–314.

Björck, I. & Asp, N. G. (1991). Balance experiments in Nebacitin-treated rats. In *Methodological Aspects of In Vivo Methods for Measurement of Starch Digestibility*, pp. 35–39 [E. Gudmand-Hoyer, editor]. Den Haag: CIP Data Koninklijke Bibliotheek.

Björck, I., Nyman, M., Pedersen, B., Siljeström, M., Asp, N.-G. & Eggum, B. O. (1986). On the digestibility of starch in wheat bread – studies *in vitro* and *in vivo*. *Journal of Cereal Sciences* **4,** 1–11.

British Nutrition Foundation (1990). *Complex Carbohydrates in Foods. The Report of the British Nutrition Foundation's Task Force.* London: Chapman & Hall.

Champ, M. (1992). Determination of resistant starch in foods and food products: interlaboratory study. *European Journal of Clinical Nutrition* **46,** Suppl. 2, S51–S62.

Champ, M., Noah, L., Loizeau, G. & Kozlowski, F. (1996). Analytical methods for resistant starch. In *Proceedings of the AOAC International Workshop on Definition and Analysis of Complex Carbohydrates/Dietary Fiber*, Nashville, USA [S. C. Lee and L. Prosky, editors]. (In the Press).

Cuber, J. C. (1982). Etude du dosage de l'amidon par voie enzymatique (Study of the enzymic determination of starch). *Sciences des Aliments* **2,** 223–232.

Dysseler, P., Hoffem, D., Fockedey, J., Quemener, B., Thibault, J. F. & Coussement, P. (1994). In *Determination of Inulin and Oligofructose in Food Products (AOAC Dietary Fibre Modified Method). Proceedings of a Workshop Physico-chemical Properties of Dietary Fibre and Effect of Processing on Micronutrients*

Availability, pp. 65–73 [R. Amado, J. L. Barry and W. Frolich, editors]. Luxembourg: Office for Official Publications of the European Communities.

Englyst, H. N. & Cummings, J. H. (1987). Digestion of polysaccharides of potato in the small intestine of man. *American Journal of Clinical Nutrition* **45,** 423–431.

Englyst, H. N. & Cummings, J. H. (1988). Improved method for measurement of dietary fiber as non-starch polysaccharides in plant foods. *Journal of the Association of Official Analytical Chemists* **71,** 808–814.

Englyst, H. N., Cummings, J. H. & Wood, R. (1987*a*). Determination of dietary fibre in cereals and cereal products – collaborative trials. II. Study of a modified Englyst procedure. *Journal of the Association of Public Analysts* **25,** 59–71.

Englyst, H. N., Cummings, J. H. & Wood, R. (1987*b*). Determination of dietary fibre in cereals and cereal products – collaborative trials. III. Study of further simplified procedure. *Journal of the Association of Public Analysts* **25,** 73–110.

Englyst, H. N. & Hudson, G. (1987). Colorimetric method for routine measurement of dietary fibre as non-starch polysaccharides. A comparison with gas–liquid chromatography. *Food Chemistry* **24,** 63–76.

Englyst, H. N., Kingman, S. M. & Cummings, J. H. (1992). Classification and measurement of nutritionally important starch fractions. *European Journal of Clinical Nutrition* **46,** Suppl. 2, S33–S50.

Englyst, H. N., Trowell, H., Southgate, D. A. T. & Cummings, J. H. (1987*c*). Dietary fiber and resistant starch. *American Journal of Clinical Nutrition* **46,** 873–874.

Englyst, H. N., Wiggins, H. S. & Cummings, J. H. (1982). Determination of the non-starch polysaccharides in plant foods by gas–liquid chromatography of constituent sugars as alditol acetates. *Analyst* **107,** 307–318.

Faisant, N., Buléon, A., Colonna, P., Molis, C., Lartigue, S., Galmiche, J. P. & Champ, M. (1995*a*). Digestion of raw banana starch in the small intestine of healthy humans: structural features of resistant starch. *British Journal of Nutrition* **73,** 111–123.

Faisant, N., Planchot, V., Kozlowski, F., Pacouret, M.-P., Colonna, P. & Champ, M. (1995*b*). Adaptation of the Berry modified method to high RS products. *Sciences des Aliments* **15,** 83–89.

Faulks, R. M. & Timms, S. B. (1985). A rapid method for determining the carbohydrate component of dietary fibre. *Food Chemistry* **17,** 273–287.

Holz, F. (1977). Automatische, enzymatischphotometrische Bestimmung des Stärkegehaltes von Cerealien (Automatic, enzymic–photometric determination of the starch content of cereals). *Landwischenschaft und Forschung* **33,** Suppl., 228–249.

Horwitz, W. (editor) (1980). In *Official Methods of Analysis of the Association of Official Analytical Chemists*. Washington, DC: AOAC.

Karkalas, J. (1985). An improved enzyme method for determination of native and modified starch. *Journal of the Science of Food and Agriculture* **36,** 1019–1027.

Langkilde, A. M. & Andersson, H. (1994). In vivo quantification of resistant starch on EURESTA reference materials using the ileostomy model. In *Proceedings of the Concluding Plenary Meeting of EURESTA*, pp. 31–32 [N. G. Asp, J. M. M. van Amelsvoort and J. G. A. J. Hautvast, editors]. Den Haag: CIP Data Koninklijke Bibliotheek.

Lee, S. C. & Prosky, L. (editors) (1996). Summary of AOAC survey on complex carbohydrates/dietary fiber. In *Proceedings of the AOAC International Workshop on Definition and Analysis of Complex Carbohydrates/Dietary Fiber*, Nashville, USA. (In the Press).

Lee, S. C., Vincent, R. W., Prosky, L., Jia, M. Y. & Min, D. B. (1996). Definition and analysis of complex carbohydrates. In *Proceedings of the AOAC International Workshop on Definition and Analysis of Complex Carbohydrates/Dietary Fiber*, Nashville, USA [S. C. Lee and L. Prosky, editors]. (In the Press).

Lineback, D., DeVries, J., Slavin, J., Stephen, A., Gordon, D., Prosky, L., Scarborough, F. E., Lee, S., Olson, B. & Clydesdale, F. (1995). Complex carbohydrates: the science and the label. *Nutrition Reviews* **53,** 186–193.

Monties, B. (1995). The determination of lignins: quantitative and qualitative approaches. In *Proceedings of a Workshop Physico-chemical Properties of Dietary Fibre and Effect of Processing on Micronutrients Availability*, pp. 87–92 [A. Sorenson, K. E. Bach Knudsen, E. Gudman-Hoyer and M. Nyman, editors]. Luxembourg: Office for Official Publications of the European Communities.

Muir, J. G., Birkett, A., Brown, I., Jones, G. & O'Dea, K. (1995). Food processing and maize variety affects amounts of starch escaping digestion in the small intestine. *American Journal of Clinical Nutrition* **61,** 82–89.

Muir, J. G. & O'Dea, K. (1992). Measurement of resistant starch: factors affecting the amount of starch escaping digestion in vitro. *American Journal of Clinical Nutrition* **56,** 123–127.

Muir, J. G. & O'Dea, K. (1993). Validation of an in vitro assay for predicting the amount of starch that escapes digestion in the small intestine of humans. *American Journal of Clinical Nutrition* **57,** 540–546.

Noah, L., Guillon, F., Bouchet, B., Buléon, A., Gallant, D. J., Colonna, P., Molis, C., Faisant, N., Galmiche, J. P. & Champ, M. (1995). In *Proceedings of the European Association for Grain Legume Research Conference*, pp. 276–277.

Prosky, L., Asp, N. G., Furda, I., DeVries, J. W., Schweizer, T. F. & Harland, B. (1984). Determination of total dietary fiber in foods and food products: interlaboratory study. *Journal of the Association of Official Analytical Chemists* **67,** 1044–1052.

Prosky, L., Asp, N. G., Schweizer, T. F., DeVries, J. W. & Furda, I. (1988). Determination of total dietary fiber in foods and food products: collaborative study. *Journal of the Association of Official Analytical Chemists* **71,** 1017–1023.

Quemener, B., Thibault, J.-F. & Coussement, P. (1994). Determination of inulin and oligofructose in food products and integration in the AOAC method for measurement of total dietary fibre. *Lebensmittel-Wissenshaft und Technologie* **27,** 125–132.

Robertson, J. B. & Van Soest, P. J. (1977). Dietary fiber estimation in concentrate feedstuffs. *Journal of Animal Science* **45,** Suppl. 1, 254–255.

Robertson, J. B. & Van Soest, P. J. (1981). The detergent system of analysis and its application to human foods. In *The Analysis of Dietary Fiber in Food*, pp. 123–158 [W. P. T. James and O. Theander, editors]. New York: Marcel Dekker.

Schols, H. A., Van Laere, K. M. J. & Voragen, A. G. J. (1995). Determination of oligosaccharides. In *Proceedings of a Workshop Physico-chemical Properties of Dietary Fibre and Effect of Processing on Micronutrients Availability*, pp. 53–60 [A. Sorensen, K. E. Bach Knudsen, E. Gudmand-Hoyer and M. Nyman, editors]. Luxembourg: Office for Official Publications of the European Communities.

Schweizer, T. F. (1984). Progress in dietary fibre analysis. *Mitteilungen aus dem Gebiete der Lebensmitteluntersuchung und Hygiene* **75,** 469–483.

Schweizer, T. F. (1986). Methoden zur Bestimmung von Nahrungsfasern (Method of determination of dietary fibres). In *Nahrungsfasern-Dietary Fibres*, pp. 53–73 [R. Amado and T. F. Schweizer, editors]. London: Academic Press.

Schweizer, T. F. (1989). Dietary fibre analysis. *Lebensmittel-Wissenshaft und Technologie* **22,** 54–59.

Schweizer, T. F., Andersson, H., Langkilde, A. M., Reimann, S. & Torsdottir, I. (1990). Nutrients excreted in ileostomy effluents after consumption of mixed diets with beans or potatoes. II: Starch, dietary fibre and sugars. *European Journal of Clinical Nutrition* **44,** 567–575.

Siljeström, M., Westerlund, E., Björck, I., Holm, J., Asp, N. G. & Theander, O. (1986). The effect of various thermal processes on dietary fibre and starch content of whole grain wheat and white flour. *Journal of Cereal Science* **4,** 315–323.

Slavin, J. L. & Marlett, J. A. (1983). Evaluation of high-performance liquid chromatography for measurement of the neutral saccharides in neutral detergent fiber. *Journal of Agricultural and Food Chemistry* **31,** 467–471.

Southgate, D. A. T. (1969). Determination of carbohydrates in foods. II. Unavailable carbohydrates. *Journal of the Science of Food and Agriculture* **20,** 331–335.

Theander, O. & Westerlund, E. (1986). Studies on dietary fiber. 3. Improved procedures for analysis of dietary fiber. *Journal of Agricultural and Food Chemistry* **34,** 330–336.

Thivend, P., Mercier, C. & Guilbot, A. (1965). Dosage de l'amidon dans les milieux complexes (Estimation of starch in complex media). *Annales de Biologie et de Biophysique* **5,** 513–526.

Van Soest, P. J. (1963). Use of detergents in the analysis of fibrous feeds. II. A rapid method for the determination of fiber and lignin. *Journal of the Association of Official Analytical Chemists* **46,** 829–835.

Van Soest, P. J. & Wine, R. H. (1967). Use of detergents in the analysis of fibrous feeds. IV. Determination of cell-wall constituents. *Journal of the Association of Official Analytical Chemists* **50,** 50–55.

Printed in Great Britain