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Determination of resistant short-chain carbohydrates (non-digestible oligosaccharides) using gas–liquid chromatography

Michael E. Quigley, Geoffrey J. Hudson, Hans N. Englyst*

Medical Research Council and University of Cambridge, Dunn Clinical Nutrition Centre, Hills Road, Cambridge, CB2 2DH, UK

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Abstract

We have proposed the term short-chain carbohydrates (SCC) for those species, other than the free sugars, that are soluble in 80% ethanol under well-defined conditions. We describe a technique for the measurement of resistant SCC (RSCC), which are not susceptible to pancreatic amylase or the brush border enzymes and therefore sometimes termed non-digestible oligosaccharides. In the procedure, alpha-glucans (starch and maltodextrins) are hydrolysed enzymatically to glucose and the non-starch polysaccharides (NSP) are precipitated in ethanol. Fructans are hydrolysed enzymatically and the monosaccharide constituents are reduced to acid-stable alditol derivatives before the remaining RSCC are hydrolysed with sulphuric acid. All the constituent sugars are measured as alditol acetate derivatives by gas–liquid chromatography. The protocol allows both the measurement of total RSCC and a separate, specific measurement of fructans. © 1999 Elsevier Science Ltd. All rights reserved.

Keywords: Resistant short-chain carbohydrates; Non-digestible oligosaccharides; Polysaccharides; Gas–liquid chromatography; Fructans

1. Introduction

Carbohydrates may be divided on the basis of degree of polymerization (DP) into monosaccharides (DP 1), oligosaccharides (DP 2 to about 10) and polysaccharides (DP about >11; IUB–IUPAC, 1982a, b). For analytical purposes, however, polysaccharides are defined and measured on the basis of the insolubility of polysaccharides in aqueous ethanol, usually 80%, v/v. All current methods for the measurement of dietary fibre (e.g. Englyst, Quigley, & Hudson, 1994; Prosky, Asp, Schweizer, De Vries, & Furda, 1988), incorporate the precipitation of polysaccharides in 80% ethanol.

The internationally accepted division between oligo- and polysaccharides at a DP of about 10 has no nutritional significance, and terms such as 'Non-Digestible Oligosaccharides' are misused when applied to preparations that contain a range of carbohydrate species ranging in DP from 1 to about 50. The classification of ethanol-soluble oligosaccharides and polysaccharides together as short-chain carbohydrates (SCC), as proposed by Englyst and Hudson (1996), avoids this misuse of the term oligosaccharides. An overall classification of dietary carbohydrates is outlined in Table 1 and forms the basis upon which the strategy for the method presented here has been developed.

In this work, a procedure has been developed for the measurement of resistant short-chain carbohydrates (RSCC) as the SCC that are not hydrolysed by pancreatic α-amylase or the brush border enzymes. Fructans may be measured separately or included in the measurement of the total RSCC as required.

2. Materials and methods

All reagents were of AnalaR grade or above. Distilled, de-ionized water was used throughout the method.

2.1. Chromatography

For injection, we used an open-top Uniliner sleeve (Thames Chromatography, catalogue no. 20315) packed with glass wool and a Carbofrit (Thames Chromatography, catalogue no. 20295) inside a sleeve adapter (Thames Chromatography, catalogue no. 20311).

* Corresponding author.

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2.2. GLC column conditions for measurement of mono- and disaccharides as alditol acetates

The following conditions were used: SGE BPX70 wide-bore capillary column (30 m×0.75 mm i.d.): initial column temperature, 170°C; injector temperature, 300°C; detector temperature, 300°C; init. time 1.5 min; ramp, 10.0°C/min; final temperature, 290°C; upper time, 5 min. Fig. 1 shows a typical chromatogram illustrating the separation of mono- and disaccharides using the SGE BPX70 column. A similar separation can be achieved using a Supelco SP2380 wide-bore capillary column (30 m×0.75 mm i.d.) and both columns are suitable for the analysis of non-starch polysaccharides (Englyst et al., 1994).

Table 1
Classification of plant food carbohydrates

Type	Components
Free sugars	Glucose, fructose, sucrose
Sugar alcohols	Sorbitol, xylitol, maltitol
Short-chain carbohydrates (SCC) (soluble in 80% ethanol)	Resistant SCC (fructo- and galactooligosaccharides pyrodextrins, Polydextrose)
	Maltodextrins
Starch	Rapidly digestible starch (RDS)
	Slowly digestible starch (SDS)
	Resistant starch (RS)
Non-starch polysaccharides (NSP)	Present as plant cell-walls (dietary fibre)
	Other NSP (gums, mucilages, any isolated NSP)

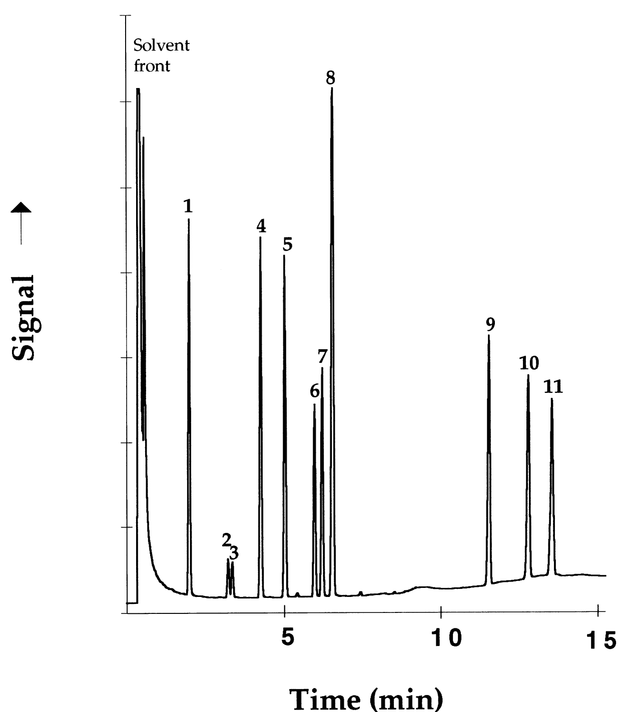

Fig. 1. Gas–liquid chromatogram of a sugar standard. Peaks: 1, i-erythritol (internal standard); 2, rhamnose; 3, fucose; 4, arabinose; 5, xylose; 6, mannose; 7, galactose; 8, glucose; 9, sucrose; 10, lactose and 11, maltose. The amount injected was 1 µl of the ethyl acetate top layer from the standard mixture taken through the reduction and derivatisation steps of the procedure as described here.

2.3. Principle

The procedure is outlined in Fig. 2. Starch and maltodextrins are hydrolysed enzymatically (enzyme solutions I and II) to free glucose before the removal of non-starch polysaccharides (NSP) by precipitation in ethanol (Englyst et al., 1994). The liquid is evaporated from a portion of the supernatant and the residual material is dissolved in acetate buffer if the analytical sample contains less than about 50 mg of starch or free glucose (e.g. some non-starchy fruits and vegetables). If the analytical sample contains large amounts of starch and/or free glucose, the residual material is dissolved in enzyme solution III (containing glucose oxidase) and the glucose is subsequently removed. Sub-sample A is removed and used for the measurement of free mono- and disaccharides. The remainder of the sample is subjected to treatment with inulinase before reduction with alkaline sodium tetraborohydrate. Sub-samples B and C are removed at this stage. Sub-sample B is used for the measurement of the monosaccharides released from fructans (calculated as B−A). Sub-sample C is subjected to acid hydrolysis and subsequently used for the measurement of the constituent sugars of all RSCC (calculated as C−A).

2.4. RSCC-containing test samples

Fructooligosaccharides and Raftiline (inulin) were from Orafti, Belgium; Fibersol-2 (pyrodextrins) was from Matsutani, Japan; Palatinose (enzymatically rearranged sucrose molecules (Palatinit) subsequently pyrolysed) was from Südzücker, Germany; Oligomate (galactans) was from Yakult, Japan; Soybean Oligosaccharides (a mixture of raffinose and stachyose) were from Calpis, Japan; Xylooligosaccharides were from Suntory, Japan; Isomaltooligosaccharides type M500 and M900 were from Showa Sangyo, Japan.

2.5. Reagents

2.5.1. Sodium acetate buffer
Sodium acetate, 0.2 mol/litre, pH 5.2, with 8 ml of calcium chloride, 1 mol/litre, added to 1 litre of buffer.

2.5.2. Enzyme solution I
Prepared as 2.5 ml of heat-stable α-amylase (Termamyl 300 LDX from Novo Nordisk) and 25 ml of internal standard (see below) made to a volume of 250 ml with the sodium acetate buffer diluted 1:1 (v/v) with

Fig. 2. A flow-chart outlining the main steps in the procedure for the analysis of RSCC by gas–liquid chromatography.

water. The solution was transferred into a positive displacement dispenser calibrated to dispense 8 ml (8000 (±10) mg) of water at 50°C, equilibrated to and kept at 50°C.

2.5.3. *Enzyme solution II*

This was prepared by placing 1.5 g of pancreatin (Sigma, catalogue number P-1500) into a 50 ml tube, adding 15 ml of water, vortex-mixed initially and then

mixed for 10 min with a magnetic stirrer. The mixture was vortex-mixed again, then centrifuged at 1500 ***g*** for 10 min. Amyloglucosidase (3 ml; Megazyme, catalogue number E-AMGDF) was added to 12 ml of the (cloudy) supernatant and vortex-mixed.

2.5.4. Enzyme solution III

This was prepared as 30 mg of catalase (Sigma, catalogue number C-40) dissolved in 1 ml of water, 3.0 ml of glucose oxidase (Merck, catalogue no. 39061 4S) and 0.3 ml of amyloglucosidase (Megazyme, catalogue no. E-AMGDF) added and the volume made to 60 ml with 0.5×sodium acetate/calcium chloride buffer.

2.5.5. Enzyme solution IV

This was prepared as 0.15 ml of inulinase (Novo Nordisk; 1800 units/g) added to 5 ml of water and mixed.

2.5.6. Internal standard

This was prepared as 37,500 mg of i-erythritol (Sigma catalogue no. E-7500; dried to constant mass under reduced pressure with phosphorus pentoxide, weighed to the nearest 1 mg), made to 1 litre with 50% saturated benzoic acid.

2.5.7. Stock sugar mixture

All sugars were dried to constant mass under reduced pressure with phosphorus pentoxide and weighed to the nearest 1 mg: 0.52 g of rhamnose, 0.48 g of fucose, 4.75 g of arabinose, 4.45 g of xylose, 2.3 g of mannose, 2.82 g of galactose, 9.4 g of glucose and 2.79 g of galacturonic acid (3.05 g of the monohydrate) were together diluted to 1 litre with 50% saturated benzoic acid. The composition of this sugar solution, which is identical with that used for the analysis of NSP by GLC (Englyst et al., 1994), was used to prepare working sugar standards 1 and 2.

2.5.8. Sugar standard 1

This was prepared by adding 100 ml of the stock sugar mixture to 500 mg of i-erythritol and 500 mg of sucrose (both dried to constant mass under reduced pressure with phosphorus pentoxide).

2.5.9. Sugar standard 2

This was prepared by adding 100 ml of the stock sugar mixture to 500 mg of i-erythritol (dried to constant mass under reduced pressure with phosphorus pentoxide).

2.5.10. Calibration of response factors

Calibration standards were prepared as follows: 4 ml of sodium acetate/calcium chloride buffer and 2.0 ml of dimethyl sulphoxide (DMSO) were added to 4 ml of sugar standard 1 (used for sub-sample A), and to 4 ml of sugar standard 2 (used for sub-samples B and C).

2.6. Analytical protocol

2.6.1. Pre-treatment of the sample

All samples were finely divided (to pass a 0.5 mm mesh) so that representative sub-samples could be taken. (Foods with a low water content ($<10\%$, w/w) can be milled, and foods with a higher water content can be homogenized wet or milled after freeze-drying.)

2.6.1.1. Step 1. Samples of between 100 and 300 mg (containing not more than 100 mg of dry RSCC) were weighed, to the nearest 0.1 mg, into a tube. Approximately 300 mg of sand (acid-washed, 50–100 mesh, Merck, catalogue no. 33094 5E) and about 15 glass balls (2.5–3.5 mm diameter, Merck, catalogue no. 33212 4G) were added to each tube.

An extra tube containing no sample was taken through the procedure as an enzyme blank.

2.6.2. Dispersion and enzymatic hydrolysis of starch

2.6.2.1. Step 2.1. Dimethyl sulphoxide (DMSO; 2 ml) was added to the sample, the tube capped, and the contents mixed immediately using a vortex mixer, treating each tube in turn. (It is important that the sample is wetted and no material is encapsulated or adhering firmly to the tube wall before proceeding.) When DMSO was added to all the tubes, the contents were vortex-mixed three or four times during a 5 min period. Then pairs of tubes were vortex-mixed and placed immediately into a boiling water-bath, removed after 20 s, vortex-mixed and immediately replaced into the bath. This was repeated for subsequent pairs of tubes until all the tubes were in the bath, where they were left for 30 min from that time. Enzyme solutions I and II were prepared during that period.

The volumes given for enzyme solutions I to IV, which were prepared immediately before use, are suitable for a batch of 24 samples.

2.6.2.2. Step 2.2. For the samples and enzyme blank, one tube at a time was removed from the boiling water-bath, vortex-mixed, uncapped and 8 ml of enzyme solution I (kept at 50°C) immediately added by positive displacement. The tube was capped and the contents vortex-mixed thoroughly, ensuring that no material was adhering firmly to the tube wall, and replaced into the boiling water-bath. This was repeated for all the tubes, which were left in the boiling water-bath (together with the working standards) for 10 min, timed from the last addition of enzyme. The tubes were transferred together into the 50°C water-bath. When the tubes were equilibrated to 50°C (approx. 3 min), 0.5 ml of enzyme solution II was added to each sample and enzyme blank, and the tube contents mixed thoroughly to aid distribution of the enzyme throughout the sample; 0.5 ml of the sodium acetate buffer diluted 1:1 (v/v) with water

was added to the standards, which were then mixed thoroughly. The tube contents were mixed after 10, 20 and 30 min. The tubes were transferred together to the boiling water-bath and left there for 10 min.

2.6.3. Separation of RSCC and NSP by precipitation of NSP

2.6.3.1. Step 3.1. The tubes were cooled in ice–water before adding 0.15 ml of hydrochloric acid, 5 mol/litre, to each. The tube contents were vortex-mixed thoroughly two or three times during a 5 min period with tubes being replaced in the ice–water. A positive displacement device was used to add 40 ml of acidified ethanol (1 ml of hydrochloric acid, 5 mol/litre, per litre of absolute ethanol) and the tube contents were mixed well by repeated inversion. The tubes were left in ice–water for 30 min then centrifuged at 1500 ***g*** for 10 min to obtain a clear supernatant liquid. Two 2-ml portions of the supernatant liquid were removed into 30 ml round-bottomed Pyrex tubes. (The residue may be used for the analysis of NSP if subjected to treatment as described from step 3.1 onwards according to the procedure of Englyst et al., 1994)

2.6.4. Treatment of ethanolic supernatant

2.6.4.1. Step 3.2. Sodium acetate (0.10 ml, 1 mol/litre) was added to the 2 ml of ethanolic supernatant from step 3.1 and vortex-mixed. The liquid was removed by evaporation in a TurboVap® LV Evaporator at 70°C with an air-pressure setting between 10 and 15 lb/in^2 (1 lb/in^2 ~6.9 kPa) and left until the samples were completely dry (approx. 20 min).

When an analytical sample contained less than 50 mg of glucose from starch and free sugars, we proceeded to step 3.2.1.1.

When an analytical sample contained more than 50 mg of glucose from starch and free sugars, we proceeded to step 3.2.1.2.

2.6.4.2. Step 3.2.1.1. Acetate buffer (2 ml) was added and the contents vortex-mixed vigorously. To serve as a sample blank (sub-sample A), 0.5 ml was removed to a glass tube, 0.5 ml of water was added and we proceeded to step 3.3 with sub-sample A. With the remainder of the sample, we proceeded to step 3.2.2.

2.6.4.3. Step 3.2.1.2. A crosshead magnetic stirrer bar (Camlab Ltd, Cambridge, UK, catalogue no. MSB.FTF10) was placed into each tube. A positive displacement device was used to add 2 ml of enzyme solution III to the samples and the enzyme blank, and 2 ml of sodium acetate buffer, 0.1 mol/litre, pH 5.2 was added to the standards; all tube contents were then vortex-mixed. The tubes were maintained at 50°C for 60 min with continuous mixing, placed together into a boiling water-bath for 10 min and then cooled to room temperature: 0.5 ml of the contents was removed into a glass tube and 0.5 ml of water added to each (these tubes (sub-sample A) were used as sample blanks) before proceeding to step 3.3. With the remainder of the sample, we proceeded to step 3.2.2.

2.6.4.4. Step 3.2.2. For hydrolysis of fructans, enzyme solution IV (0.1 ml) was added to the remaining samples and to the enzyme blank, and mixed thoroughly. Water (0.1 ml) was added to the standards, and mixed thoroughly. The tubes were maintained at 50°C for 30 min with continuous mixing then cooled to room temperature before proceeding to step 3.3.

2.6.4.5. Step 3.3. Reduction I. The tubes (including sub-sample A from step 3.2.1.1 or step 3.2.1.2) were placed into ice–water, 0.1 ml of ammonium hydroxide solution, 12 mol/litre was added, and the tube contents vortex-mixed.

A check was made to ensure that the pH of the solution was at 9 or above (a little more ammonium solution was added when required). A drop (~5 μl) of the surfactant, octan-2-ol, and 0.1 ml of freshly prepared ammonium/sodium borohydride solution (ammonia solution, 6 mol/litre, containing $NaBH_4$, 200 mg/ml) were added and the tube contents vortex-mixed. The tubes were left in a water-bath at 40°C for 30 min, then removed, and 0.2 ml of glacial acetic acid added and the tube contents vortex-mixed.

Sub-sample A: 0.5 ml of the reduced samples was removed into a glass tube before proceeding to step 3.5.

Sub-sample B: 0.5 ml was removed into a glass tube, 0.5 ml of water was added and the tube contents vortex-mixed before proceeding to step 3.5.

2.6.4.6. Step 3.4. Acid hydrolysis of non-fructan RSCC: sub-sample C: 0.2 ml of sulphuric acid, 12 mol/litre (Merck, catalogue no. 19321 6Y), was added to 1.0 ml of the remaining reduced and acidified samples from step 3.3, and the contents vortex-mixed. These tubes, which were used for measuring total RSCC (see later), were placed into a boiling water-bath, left for 30 min, and then cooled before proceeding to step 3.4.1.

2.6.4.7. Step 3.4.1. Reduction II. Sub-sample C: the tubes were placed into ice–water, 0.7 ml of ammonium solution, 12 mol/litre, added and the contents vortex-mixed. A check was made to determine that the solution was at pH 10 or above (a little more ammonium solution was added when required), 0.1 ml of the ammonium/sodium borohydride solution was added and the tube contents vortex-mixed. The tubes were placed into a water-bath at 40°C and left for 30 min. The tubes were removed from the water-bath, 0.3 ml of glacial acetic acid added and the contents mixed. A check was made to determine that the pH of the solution was between 4

and 5 (acetic acid was added 0.05 ml at a time when required) before transferring 0.5 ml to 30 ml glass tubes and proceeding to step 3.5.

2.6.4.8. Step 3.5. Acetylation. A portion (0.5 ml) of the reduced samples and standards from steps 3.3 and 3.4 was taken; 0.5 ml of 1-methylimidazole was added, followed by 5 ml of acetic anhydride and the tube contents vortex-mixed immediately. The tubes were left for 10 min before adding 0.8 ml of absolute ethanol. The tube contents were vortex-mixed and left for 5 min; 10 ml of water was added followed by vortex-mixing and the tubes were left for 5 min before adding 0.5 ml of bromophenol blue, 0.4 g/litre. The tubes were placed into ice–water and 5 ml of potassium hydroxide, 7.5 mol/litre, added; a few minutes later a further 5 ml of potassium hydroxide, 7.5 mol/litre, was added, then the tubes were capped and mixed by inversion. The tubes were centrifuged for a few minutes or left until the separation into two phases was complete (10–15 min). Part of the upper phase was drawn into the tip of an automatic pipette, avoiding the inclusion of the blue phase, and transferred to a small (autoinjector) vial. Conventional GLC measurement of the neutral sugars is described under Chromatography.

2.7. Calculations

The following ratios (from Englyst et al., 1994) were used for calibration of response factors:

Sugar	Sugar standard 1	Sugar standard 2
Rhamnose	0.52	0.52
Fucose	0.48	0.48
Arabinose	4.75	4.75
Xylose	4.45	4.45
Mannose	2.30	2.30
Galactose	2.82	2.82
Glucose	9.4	9.4
i-Erythritol (int. std.)	5.0	5.0
Sucrose	5.0	–

Sugar standard 1, which is taken through the procedure in parallel with the samples and enzyme blank, was used for calibration of response factors for sub-sample A.

Sugar standard 2 was used for calibration of response factors for monosaccharides released from polysaccharides in sub-samples B and C.

The amount of each sugar (expressed as g per 100 g of sample) is calculated as:

$$\frac{A_T \times M_I \times 100 \times R_F}{A_I \times M_T}$$

where A_T and A_I are the peak areas of the test sample and internal standard, respectively, in sub-samples A, B and C; M_T is the mass (in mg) of the test sample; M_I is the mass (in mg; here 30) of the internal standard; R_F is the response factor for individual sugars obtained from the calibration run with sugar standard 1 (sub-sample A only) and sugar standard 2 (sub-samples B and C) treated in parallel with the samples. After deducting values for the enzyme blank (adjusted for individual sample weights) the total fructans RSCC or the value for total RSCC fructans in the sample were calculated.

2.7.1. Calculation of fructans

The amount of fructans (expressed as g per 100 g of sample) is calculated as:

$$\mathrm{RSCC_F} = (T_B - T_A) \times F$$

where T_B is the total carbohydrate in sub-sample B after inulinase treatment; T_A is the sum of the mono- and disaccharides in the sample blank, sub-sample A; F is the factor for converting the experimentally determined values for monosaccharides to short-chain carbohydrates. For samples of unknown DP or where average DP is 5, $F = 0.92$; for disaccharides, $F = 0.95$; for tri- and tetrasaccharides, $F = 0.93$; and for mixtures containing mostly polysaccharides, $F = 0.90$.

2.7.2. Calculation of total RSCC

The total amount of RSCC (expressed as g per 100 g of sample) is calculated as:

$$\mathrm{RSCC_T} = (T_C - T_A) \times F$$

where T_C is the total sugars in batch C after acid hydrolysis; and T_A is the sum of the mono- and disaccharides in the sample blank, sub-sample A (note the sucrose can be deducted directly from the total or as monosaccharides that would be released from the sucrose as glucose and fructose. The released glucose is measured as glucitol, and we found that the released fructose is measured as glucitol (~50%) and as mannitol (~50%). Therefore, 75% of sucrose is measured as glucitol and the remaining 25% as mannitol.)

F is the factor for converting the experimentally determined values for monosaccharides to RSCC as described above.

3. Results and discussion

3.1. Optimisation of starch hydrolysis

The starch enzymatic hydrolysis procedure is the same as that used in the preparation of samples for the measurement of dietary fibre as non-starch polysaccharides

(NSP; Englyst et al., 1994; Pendlington, Meuree-Vanlaethem, & Brookes, 1996; Wood, Englyst, Southgate, & Cummings, 1993), except that, here, amyloglucosidase has been introduced to ensure that starch and maltodextrins are completely hydrolysed to glucose, which is not required for the measurement of NSP.

3.2. Solubility of RSCC preparations in ethanol

Samples of the RSCC preparations were taken through the starch hydrolysis procedure (see Materials and methods) and ethanol was added to final concentrations of between 50 and 90% (v/v). All the preparations were less soluble in 90% than in 80% ethanol (data not shown). There was no difference in solubility between 50 and 80% ethanol for any of the samples except Fibersol-2, which was 91.7% soluble in 50% ethanol but only 39.3% soluble in 80% ethanol.

The effect of sample amount on the recovery of a range of RSCC preparations taken through the procedure up to and including precipitation in 80% ethanol at pH 2 is shown in Table 2. There was little difference in recovery for sample amounts up to 200 mg.

3.3. Conversion of glucose to gluconic acid using glucose oxidase

The time-course for conversion to gluconic acid (step 3.2.1) of an amount of glucose corresponding to a sample weight of between 50 and 300 mg was determined. The optimised amounts of glucose oxidase (GOD) and catalase, as used in the procedure, result in virtually complete conversion of glucose after 60 min with continuous vigorous mixing using a magnetic stirrer-bar (data not shown). To obtain quantitative measurements following the treatment with GOD it is important that the pH of the hydrolysate is 10 or above before the addition of the ammonium solution–sodium borohydride solution (at lower pH values, 7–9, a glucose peak appears that seems to be the result of a pH-dependent equilibrium between glucose and gluconic acid). The GOD step is used to avoid exceeding the linear detection limit of the detector, which may occur if there is a large amount of starch or free glucose in the sample.

3.4. Optimisation of RSCC hydrolysis conditions

Fructooligosaccharides and inulin (Raftiline) were taken through the procedure (Fig. 2) and the optimal amount of inulinase required for complete hydrolysis in step 3.2.2 was determined (data not shown). To determine the recovery of free and bound fructose after the enzymatic and acid hydrolysis steps, samples of fructose, sucrose, Fructooligosaccharides and inulin (Raftiline) were subjected to: (1) treatment with sulphuric acid, 2 mol/litre, at 100°C for 30 min followed by reduction I; (2) treatment with inulinase followed by reduction I; and (3) treatment with inulinase, followed by reduction I, followed by treatment with sulphuric acid, 2 mol/litre, at 100°C for 30 min, followed by reduction II. All the reduced samples were subjected to derivatisation as described in step 3.5 (see Materials and methods). Table 3 shows that the recovery of fructans subjected to acid hydrolysis (treatment 1) is low, ranging from 20.6% for fructose to 51.9% for sucrose, reflecting the fact that only the fructose moiety of sucrose is labile under these acid hydrolysis conditions. When the samples were subjected to treatments 2 or 3, the observed recoveries represented the expected values for fructose and the fructans. In treatment 3, fructans are hydrolysed enzymatically to free fructose, which is reduced to the acid-stable alditol before the treatment with acid required to hydrolyse the non-fructan RSCC, resulting in the near-complete recovery observed.

Table 4 shows the recovery of a range of RSCC preparations subjected to treatment with sulphuric acid, 2 mol/litre, at 100°C for 30 and 60 min. Recoveries were not increased at 60 min and therefore 30 min of treatment is incorporated into the procedure. The combination of enzymatic hydrolysis and reduction followed by acid hydrolysis as described here allows all of the RSCC to be measured in a single chromatographic run, or for the fructans to be measured separately if required.

Table 2
Effect of sample size. Total carbohydrates soluble in 80% (v/v) ethanol from various preparations taken through the starch hydrolysis procedure and ethanolic precipitation steps. Values are average (duplicate analyses) recoveries

Preparation	Recovery (g/100 g as received)				
	Sample amount (mg)				
	50	100	200	300	400
Fructooligosaccharides	91 (±1.6)	90 (±3.4)	91 (±2.1)	89 (±0.2)	84 (±3.0)
Polydextrose	73 (±1.6)	68 (±1.5)	58 (±0.6)	47 (±2.2)	37 (±2.4)
Fibersol-2	42 (±1.4)	40 (±0.5)	37 (±0.5)	34 (±0.5)	31 (±0.7)
Soya oligosaccharides	40 (±1.4)	42 (±3.3)	43 (±1.5)	41 (±0.8)	40 (±1.4)
Xylooligosaccharides	86 (±0.6)	90 (±0.2)	87 (±0.3)	81 (±1.6)	37 (±2.2)

Table 3
Recovery of fructose (measured as mannose and glucose) from a range of samples after: acid hydrolysis (treatment 1); treatment with inulinase (treatment 2); treatment with inulinase followed by reduction of released fructose to alditols, followed by acid hydrolysis (treatment 3). Values are mean (standard deviation) of three analyses

Sample	Treatment	Monosaccharide (g/100 g as received)		
		Man	Glc	Total
Fructose	1	10.4 (0.2)	10.2 (0.5)	20.6 (0.6)
	2	46.3 (0.8)	49.6 (0.5)	95.8 (1.4)
	3	43.7 (0.9)	48.6 (0.3)	92.3 (1.2)
Sucrose	1	5.9 (0.2)	46.0 (0.3)	51.9 (0.5)
	2	24.9 (0.6)	69.0 (2.7)	93.9 (2.9)
	3	23.2 (0.3)	69.4 (0.6)	92.6 (0.8)
Fructooligosaccharides	1	10.6 (0.8)	22.9 (1.1)	33.4 (1.7)
	2	39.3 (0.4)	54.1 (3.2)	93.4 (2.8)
	3	37.9 (0.1)	58.1 (0.9)	96.0 (0.9)
Raftiline	1	10.3 (0.5)	19.0 (0.7)	29.3 (1.1)
	2	40.6 (1.0)	51.2 (0.3)	91.8 (1.2)
	3	41.1 (1.2)	52.8 (2.4)	93.9 (3.3)

Table 4
Values for total neutral sugars in a range of preparations obtained after treatment with sulphuric acid, 2 mol/litre at 100°C for 30 and 60 min. Values are mean (standard deviation) of three analyses

Sample	Time (min)	Monosaccharide (g/100 g as received)					
		Ara	Xyl	Man	Gal	Glc	Total
Polydextrose	30	0.1 (0)	0.1 (0)	0.2 (0)	–	85.0 (2.2)	85.4 (2.2)
	60	0.1 (0)	0.1 (0)	0.2 (0)	–	86.1 (0.3)	86.4 (1.3)
Fibersol-2	30	0.2 (0.1)	0.1 (0)	0.2 (0)	0.1 (0)	87.1 (0.9)	87.5 (0.8)
	60	0.2 (0)	0.1 (0)	0.2 (0)	0.1 (0)	85.6 (0.8)	86.0 (0.8)
Xylooligosaccharides	30	2.1 (0.2)	37.2 (1.1)	0.2 (0.1)	0.1 (0)	47.2 (2.4)	86.8 (3.7)
	60	2.0 (0)	35.6 (0.3)	0.1 (0)	0.1 (0)	45.8 (1.3)	83.7 (1.5)
Isomaltooligosaccharides	30	–	–	–	–	62.1 (0.2)	62.1 (0.2)
(type M900)	60	–	–	–	–	60.3 (2.4)	60.3 (2.4)
Oligomate	30	0.2 (0)	0.1 (0)	–	31.9 (0.7)	35.1 (0.2)	67.3 (0.7)
	60	0.2 (0)	0.1 (0)	–	31.6 (1.3)	34.4 (0.9)	66.4 (2.2)

3.5. Measurement of RSCC in the presence of glucose and starch

To demonstrate that the technique described here can be applied in the presence of large amounts of glucose and starch, RSCC were measured for the Fructooligosaccharides preparation with and without addition of glucose or potato starch (Table 5). No difference in values for Fructooligosaccharides was obtained, indicating that the GOD step is very efficient in removing free glucose.

3.6. RSCC in a range of RSCC preparations

Table 6 shows the constituent sugars and the total RSCC values for a range of commercially available RSCC-containing preparations. The total RSCC measured in these samples ranges from 20.0 g per 100 g in Fibersol-2 to 87.4 g per 100 g for Fructooligosaccharides. The remaining material was identified by supplementary analyses; e.g. the Fructooligosaccharides preparation contained 6% water, 5.8% free sugars (glucose, fructose and sucrose) and 87.4% total RSCC. There are considerable amounts of free monosaccharides, starch and NSP in the other RSCC-containing preparations which, when added to the RSCC values given here (Table 6), bring the total carbohydrates close to the total obtained after direct acid hydrolysis. For example, the Xylooligosaccharides preparation contained 29.4% RSCC, 41.0% starch, 15.0% monosaccharides and 0.3% NSP. The sum of these carbohydrate fractions is 85.8 g/100 g vs the value of 86.8 g per 100 g found after direct acid hydrolysis (Table 4).

4. Conclusions

There is a variety of established methods for the measurement of individual free sugars, starch and

Table 5
Total resistant short-chain carbohydrates in Fructooligosaccharides in the presence and absence of glucose or potato starch. Values are mean (standard deviation) of three analyses

	Monosaccharide (g/100 g as received)					
	Ara	Xyl	Man	Gal	Glc	Total
Fructooligosaccharides	–	–	35.7 (1.7)	–	58.0 (1.5)	93.7
Fructooligosaccharides + glucose	–	–	35.4 (0.4)	–	57.2 (0.6)	92.6
Fructooligosaccharides + starch	–	–	34.3 (0.5)	–	59.1 (0.6)	93.4

Table 6
Total RSCC in a range of preparations. Values are mean (standard deviation) of three analyses

Sample	Dry matter	Monosaccharide (g/100 g as received)					
	(g/100 g)	Ara	Xyl	Man	Gal	Glc	Total
Fructooligosaccharides	94.0	–	–	34.0 (0.7)	0.2 (0)	53.3 (0.6)	87.4 (1.3)
Isomaltooligo- (M500)	74.5	–	–	–	–	21.1 (1.8)	21.1 (1.8)
Isomaltooligo- (M900)	77.8	–	–	–	–	29.8 (0.5)	29.8 (0.5)
Oligomate	74.9	0.1 (0.1)	–	0.8 (0.3)	18.6 (0.8)	22.7 (1.8)	42.2 (2.5)
Palatinose	92.6	0.1 (0)	–	10.5 (0.2)	–	35.7 (2.9)	46.3 (2.8)
Polydextrose	89.8	–	–	0.3 (0.3)	1.9 (0.2)	36.5 (2.6)	38.7 (2.6)
Fibersol-2	94.3	–	–	–	0.2 (0)	18.8 (0.5)	20.0 (0.5)
Raftiline	93.3	–	0.1 (0.0)	34.7 (2.0)	0.8 (0.5)	50.2 (1.9)	85.8 (3.4)
Soybean oligosaccharides	76.5	–	0.1 (0)	7.5 (0.6)	8.4 (1.1)	15.6 (1.6)	32.2 (3.9)
Xylooligosaccharides	94.9	0.8 (0.3)	25.9 (0.7)	0.6 (0.4)	–	1.6 (0.3)	29.4 (1.3)

'dietary fibre', the latter all designed to include non-starch polysaccharides. To date, however, the remaining fraction of dietary carbohydrates, which we have chosen to term the short-chain carbohydrates (SCC), has been largely ignored.

The method described for the measurement of RSCC, some of which are being used as prebiotics (Gibson, Beatty, Wang, & Cummings, 1995; Roberfroid, Bornet, Bouley, & Cummings, 1995; Van Loo, Coussement, De Leenheer, Hoebregs, & Smits, 1995), forms part of an overall analytical strategy (Table 1) for measurement of total carbohydrate as the sum of its components.

The technique described here represents an important advance in the development of the nutritional classification of dietary carbohydrates and the establishment of techniques for their specific measurement. The GLC technique for the measurement of RSCC, described here, and our recently developed HPLC technique for the measurement of total SCC (to be described in detail elsewhere) complete the spectrum of methodology for the direct and specific measurement of all nutritionally important fractions of dietary carbohydrates identified to date.

The classification scheme is flexible and new methods may need to be developed in response to advances in technology and the acquisition of new knowledge of the links between dietary carbohydrates and health. At present, however, we now have the means to measure dietary carbohydrates as the sum of their parts by direct and specific measurement of nutritionally important fractions. The same range of analytical tools will serve in the investigation of the mechanisms underlying the links between dietary carbohydrates and health, and for the interpretation of epidemiological data.

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