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A nutritionally valid procedure for measuring soluble dietary fibre

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The need for nutritional relevance and standardization in dietary-fibre analysis is discussed. Analytical conditions that simulate the gastrointestinal environment are proposed for a nutritionally valid method for soluble-fibre analysis. The method differs from others currently in use by extracting soluble fibre before and separately from starch digestion and by using sequential gastric and intestinal media. Comparison of the proposed method with a standard procedure showed that it extracted less fibre, was sensitive to the effects of cooking, and was more similar in degree of fibre extraction to in-vivo conditions (in the rat model) than the standard method. It was also highly sensitive to pH. It is concluded that current methods of soluble fibre measurement lack nutritional validity, that priority must be given to valid measurement of soluble fibre rather than to starch digestion, that conditions should be dictated by the gut environment and not by analysts, and that conditions must be strictly standardized.

INTRODUCTION

Methods for the measurement of dietary fibre have historically given priority to eliminating starch as an early step in the analytical sequence. Soluble fibre is almost invariably measured as the fibre fraction extracted under the conditions chosen for starch gelatinization and digestion (e.g. Asp *et al.*, 1983; Mongeau & Brassard, 1986; Theander & Westerlund, 1986; Brillouet *et al.*, 1988; Englyst & Cummings, 1988; Prosky *et al.*, 1988). These conditions typically include hot buffer (100°C) with thermostable amylase, and do not resemble the gastrointestinal (GI) environment. Measurements from current methods for analysing soluble fibre cannot therefore be claimed to represent fibre soluble in the GI tract and, as such, are of limited nutritional relevance. In addition, the conditions chosen for starch removal differ considerably from method to method. This results in large differences in the extraction of certain classes of fibre polysaccharide, such as pectic substances (Monro, 1991), and to discrepancies between the methods in the soluble/insoluble fibre values that they provide (Graham *et al.*, 1988; Marlett *et al.*, 1989; Ravindran & Palmer, 1990).

Uncertainty surrounding the nutritional relevance of the results is therefore compounded by a lack of standardization and, although fibre methods are being continually developed, the emphasis has been on increasing their reliability and convenience, with less attention to the nutritional meaning of the soluble fibre measurements that they provide. Given the extent to which such data are relied on by nutritionists and dietitians, this is a serious problem. Numerous studies in clinical nutrition (e.g. Cara *et al.*, 1992; Kashtan *et al.*, 1992), for instance, have used 'soluble' fibre measured by standard methods as an independent variable, even though the soluble fibre that influenced the physiologically dependent variables was that which was soluble in the GI tract, and not that which was soluble under the analytical conditions used.

Although the desirability of physiological relevance has been mentioned from time to time, little has been done to address the problem. Methods have been used that are purportedly physiological, but seldom with any commitment to faithful simulation of in-vivo conditions. For instance, the prototype 'physiological' method of Schweizer and Wursch (1979) employed HCl/pepsin followed by pancreatin, but the HCl/pepsin was at pH 1·5, 37°C for 20 h and the pancreatin was in phosphate buffer, 37°C for 18 h. Furthermore, it was recommended that starch-containing products be autoclaved for 1 h at 130°C to ensure complete gelatinization of starch. In fact, nearly all of the wide range of published methods for analysing soluble fibre deviate widely at some point from the natural conditions to which the fibre complex would be exposed *in vivo* (Monro, 1991).

As well as reducing the nutritional relevance of the soluble-fibre analysis, methods involving aqueous heat

treatments are likely also to obliterate the distinction between raw and cooked foods, so that they are not suitable for the measurement of the actual soluble fibre load contributed in the diet. Nonetheless, considerable effort has been expended on analysing soluble fibre in raw and cooked foods using hot phosphate buffer (Englyst *et al.*, 1988), even though this treatment effectively strips pectic substances, which are susceptible to cooking (van Buren, 1979), from pectin-rich foods such as fruit and vegetables (Monro, 1991).

While starch removal is an essential part of any fibre method, if the objective is to measure soluble fibre, fibre extraction must be given priority rather than being grafted inappropriately into a total fibre method. The fibre must also be extracted under conditions as near as possible to those encountered *in vivo*, with starch removal as a secondary step. Only then can the meaning of the term 'soluble fibre' become 'fibre potentially soluble in the upper GI tract', rather than 'fibre extracted under the particular conditions used for starch gelatinization and digestion'. Until there is a common set of conditions linking solubility of fibre during analysis *in vivo*, to its solubility *in vitro*, results will have little nutritional validity; and if food analysts could accept an agreed-upon set of representative GI conditions, the problems of nutritional relevance and standardization would be largely solved.

An 'ideal' set of in-vitro, GI conditions can be derived from literature values. Gastric and intestinal pH profiles have been measured in healthy ambulatory humans using anchored in-situ electrodes (McLaughlan *et al.*, 1989) or radiotelemetry capsules (Evans *et al.*, 1988). Gastric pH and gastric emptying have also been measured in the presence and absence of dietary fibre (Tadesse, 1986). Such studies have shown that gastric pH varies between about 1·5 and 4·5, with a mean of about 2·0. In the case of radiotelemetry capsules consumed with a meal, the residence time in the stomach was less than 2 h, and the distal small bowel was reached 3–4 h after exit from the stomach and it was uniformly near-neutral in pH.

A reasonable approximation to conditions in the GI tract would involve exposure to HCl/NaCl-pepsin pH 2·0, 37°C, 2 h, followed by exposure to intestinal buffer, pH 7·0, 2 h On this basis, a more nutritionally valid procedure for soluble-fibre measurement is proposed here, and is compared with the standard procedure of Englyst and Cummings (1988), which is similar to the AOAC method of Prosky *et al.* (1988) in its conditions and degree of extraction of soluble fibre.

MATERIALS AND METHODS

Source and treatment of food samples

Foods were purchased at a local supermarket and were in sound condition. Edible portions were removed, cooked where appropriate, immediately frozen, and freeze-dried. The freeze-dried foods were ground to a powder in a coffee mill with care taken to avoid heating. Sub-samples were stored frozen over desiccant in sealed containers until analysis.

A simulated gastrointestinal (GI) method for soluble-fibre measurement

Extractions were carried out in duplicate in 15-ml Falcon tubes with 100–200 mg of accurately weighed, freeze-dried, powdered sample, and involved sequential extraction of the samples by rotating the tubes at 37°C with gastric and, subsequently, intestinal media. Gastric medium was the 0·1 M HCl/54 mM NaCl mixture used by Edwards *et al.* (1987). Gastric medium (12 ml, 0·1 M, pH 2·0) was added to the tubes. The tubes were then shaken for 10 min and centrifuged. This preliminary wash was required to ensure that the pH of the gastric medium was maintained after addition to the sample. The residue was incubated (2 h) in a further 12 ml of the HCl/NaCl medium containing 20 mg pepsin (1:10000; Sigma P7000), adjusted to pH 2·0 if necessary. After incubation, the tubes were centrifuged, and the residue was washed with intestinal buffer (10 ml, pH 7·0), centrifuged, and then incubated for 2 h with a further 10 ml of the intestinal buffer containing pancreatin (Sigma P7545: 0·5% (w/v); centrifuged supernatant). Prolonging the intestinal digestion to 4 h did not greatly enhance the extraction of soluble fibre. The intestinal buffer was based on the Tyrode buffer of Platt and Clydesdale (1987) and contained, per litre, NaCl (8·0 g), KCl (0·2 g), $MgSO_4 7H_2O$ (0·26 g), $NaH_2PO_4 2H_2O$ (0·06 g), $NaHCO_3$ (1·0 g) and $CaCl_2$

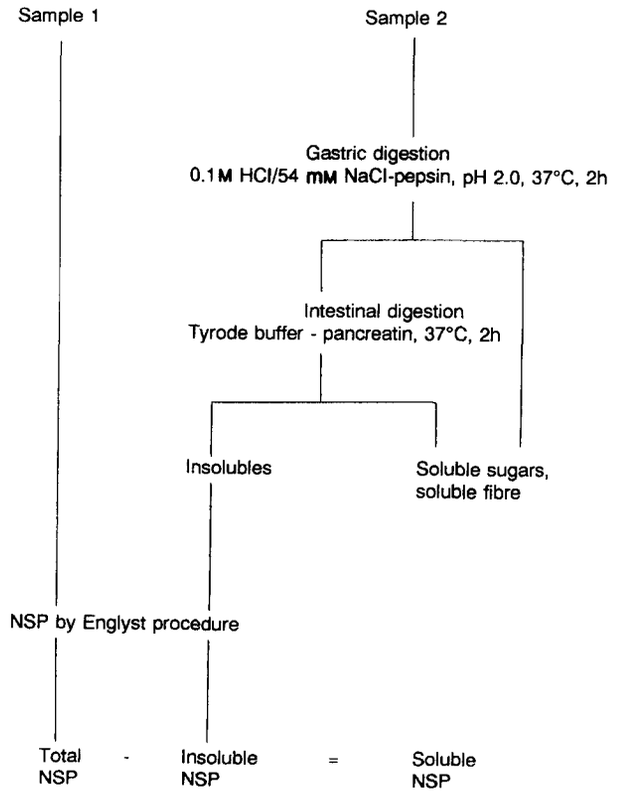

Fig. 1. Summary flow chart of procedure for nutritionally valid measurement of soluble fibre.

(0·2 g). The tubes were centrifuged, the residue was washed with intestinal buffer (15 ml) and then freeze-dried. Non-starch polysaccharide (NSP) in the residue was then measured using the Englyst procedure for total NSP. Total NSP was measured in samples run in parallel without the GI extraction. Soluble fibre was calculated from the difference between NSP with and without the GI extraction. Alternatively, soluble fibre may be precipitated from the combined washes and digests with 80% ethanol. The procedure is summarized in Fig.1.

Time course of fibre extraction with phosphate buffer and gastric medium

Extracts of dried powdered carrot (100 mg) were carried out as above except that the extraction media were (a) phosphate buffer, 0·2 M, pH 7·0, 100°C, (b) phosphate buffer, 0·2 M, pH 7·0, 37°C, and (c) gastric medium, pH 2·0, 37°C. With each medium, extractions were for 10, 20, 30, 40, 60, 80, 120 and 150 min. The tubes were then immediately cooled in iced water for 1 min, centrifuged (2000 *g*, 1 min) and the residue quickly resuspended in 10 ml of ice-cold medium with which the sample had been extracted, and again collected by centrifugation. The tubes were placed in a freeze-drier and the dried residues were subsequently analysed for total NSP using the Englyst procedure in the same tubes.

Sensitivity of GI and Englyst procedures to prior cooking of samples

Samples (10 g) of raw cauliflower and carrot were diced to about 0·5 cm^3. Half of each sample was immediately frozen, and the other half was placed in a covered beaker and heated in a microwave oven. The heated samples were maintained at 100°C in a boiling water bath for 20 min. Cooking was stopped by placing the tubes in iced water and then adding 5 ml of iced water to the samples. Oatmeal and ground barley samples were cooked by adding 20 ml of boiling water to 2 g of the dry sample in a flask, and maintaining the slurry at 100°C for 20 min with occasional stirring. The flasks were then placed in iced water and the contents stirred until cool. All samples were then frozen, freeze-dried, and ground prior to analysis. Soluble fibre in the samples was then measured in triplicate by the GI and Englyst procedures.

Effect of pH on the time course of pectin extraction from carrot by gastric medium

Ground carrot samples (250 mg) were washed with 50 ml 80% ethanol 10 min, 40°C (to reduce interference by soluble sugars with polyuronide measurement), collected by centrifugation, and added to 50 ml gastric medium at pH 1·5, 2·0, 2·5, 3·0, 3·5, and 7·0. The flasks were shaken and aliquots of the homogeneous slurry were taken for each pH at 0·5, 1, 2, 3, 4 and 5 h. The aliquots were centrifuged and pectin in the supernatant was measured directly as polyuronide using the method of Scott (1979).

Effect of pH of gastric medium on extraction of soluble fibre

The measurement of GI-soluble fibre was carried out as above, but the pH of the gastric medium used was adjusted to pH 1·5, 2·0, 2·5, 3·0 and 7·0 by adding HCl or $NaHCO_3$ from a glass rod, as appropriate, prior to extracting the soluble fibre. The pH of the tubes was checked at the end of the gastric incubation period.

A comparison of soluble-fibre extraction using the Englyst, simulated-GI and in-vivo conditions in the rat

Diets containing native soluble fibre were prepared by mixing freeze-dried ground celery, cauliflower, carrot, barley, and oatbran, each with ground, standard rat pellets. Four male Spague-Dawley rats (200–250 g) were randomly assigned to each diet. The rats were individually housed in wire-bottomed cages with cage position randomized in a controlled-environment house. The rats were then meal-fed the powdered diets for 2 h daily (8–10 am) for a period of 1 week, with food intake measured daily. Four hours after completion of the final meal the rats were slaughtered by CO_2 asphyxiation, and the GI tract was removed. The stomach contents were weighed, thoroughly mixed with four times their weight of ice-cold distilled water, and 20 ml of the resulting slurry centrifuged. The pellets were washed with distilled water in a further 20 ml volume. After again centrifuging, the pellets were freeze-dried. Total and insoluble fibre remaining in the washed gastric contents was measured using the Englyst method. Samples of the feeds were analysed using the Englyst and simulated-GI procedures outlined above, to determine total and insoluble fibre contents. The soluble fibre extracted under each set of conditions was then calculated by difference. The polysaccharide remaining after the Englyst extraction is essentially hemicellulose plus cellulose, and this was used as a base in comparing the degrees of fibre extraction under the Englyst, simulated-GI, and in-vivo conditions respectively. It was assumed that the insoluble fibre measured using the Englyst procedure was not significantly solubilized under the in-vivo conditions; work in this laboratory has shown that most of the pectic polysaccharide is extracted using the Englyst procedure (Monro, 1991) and that simulated GI conditions extract little further polysaccharide from a range of foods that have been subjected to the Englyst soluble-fibre extraction.

Measuring polysaccharide in the residues

Non-starch polysaccharide in all residues was measured as reducing sugar after acid hydrolysis. The residues were dispersed in 12 M H_2SO_4, (2 ml, 30°C, 1 h), distilled water (10 ml) added, and the capped tubes heated

(100°C) for 1 h. Reducing sugars in the hydrolysates were measured manually using the dinitrosalicylate (DNS) method, as described by Englyst and Hudson (1987), except that all volumes were halved. Polyuronide was measured as uronic acid in hydrolysates by the 3,5-dimethylphenol method of Scott (1979) as used by Englyst and Hudson (1987), using a D-galacturonic acid standard. A non-starch polysaccharide standard, consisting of cellulose: xylan: arabinogalactan: pectin (1:1:1:1) was run in parallel during the acid hydrolysis.

RESULTS AND DISCUSSION

This paper is concerned with a basic change in methodology—extraction of soluble fibre under physiological conditions *prior* to starch removal—required to bring soluble-fibre measurements in line with human biology. It does not address all of the problems associated with relevance, such as the use of freeze-dried samples rather than samples as consumed. Freeze-dried samples were simply used for convenience in the present study.

Comparative values for fibre extracted under simulated-GI and under Englyst's fibre-analysis conditions are shown in Table 1. The comparison would also be valid for the Prosky method (Prosky *et al.*, 1988) which is similar to the Englyst method in the use of hot phosphate buffer and in the amounts of soluble fibre that it extracts. The higher soluble-fibre values from Englyst's procedure are particularly marked in the case of fruit and vegetables, and less so in the case of cereals.

A time course of extraction (Fig. 2) shows an immediate powerful effect of hot phosphate buffer, and a much slower effect of GI conditions. Cold phosphate buffer extracted less than the gastric medium and much less than hot phosphate buffer, suggesting that a combination of heat plus phosphate is responsible for the inflated soluble-fibre values from the Englyst (and Prosky) methods. A similar difference between hot phosphate and hot acetate buffers in measuring soluble fibre has been reported and attributed to the destabilization of polyuronide associations, by phosphate removing Ca^{2+} from junction zones within the pectin network (Monro, 1991).

The effect of pH of the gastric medium on pectic polyuronide extraction from carrot as a function of time (Fig. 3), suggests that the pectin fraction may be

Table 1. Soluble-fibre extraction (% total fibre) under conditions of Englyst and simulated-GI procedures

	Englyst	GI
Barley	42	26
Oatmeal	43	27
Peas (frozen)	31	20
Cauliflower	55	34
Carrot	58	37
Orange	68	43

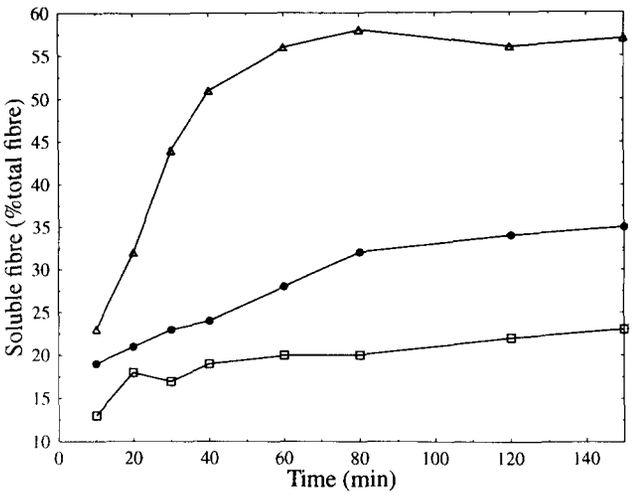

Fig. 2. Time course of soluble-fibre extraction from carrot powder by phosphate buffer, 0·2M, pH 7·0 at 100°C (Δ) and 37°C (□), and by gastric medium, 37°C (●).

responsible for the susceptibility of foods such as fruit and vegetables to conditions of fibre analysis. The ability of gastric buffer to extract soluble fibre was very dependent upon pH (Fig. 4). Extraction decreased sharply with rising pH, particularly in the case of carrots, cabbage, and orange.

Table 2 shows the effect of cooking duration on soluble fibre measured using the simulated-GI or Englyst method. Under GI conditions, more soluble fibre was extracted as cooking time increased, whereas under Englyst's conditions soluble fibre was extracted almost immediately and totally—cooking effects were largely obliterated under Englyst's conditions. Measuring soluble fibre under simulated-GI conditions may therefore allow sensitivity to the effects of food processing on actual soluble fibre load to be achieved. Although insensitivity to the effects of food processing may be seen as an advantage by the food analyst, the extensive soluble-fibre measurements made on raw and cooked foods using Englyst's procedure (Englyst *et al.*,

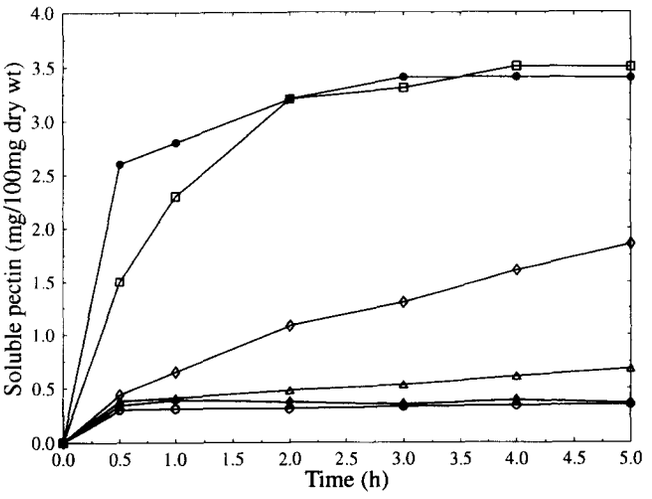

Fig. 3. Effect of pH on time course of pectin extraction from carrot power in gastric medium (54mM NaCl adjusted to required pH by addition of conc. HCl or NaHCO3). pH 1·5 (●), 2·0 (□), 2·5 (◇), 3·0 (Δ), 3·5 (○), 7·0 (◆).

Table 2. Effects of cooking duration on soluble-fibre extraction (% total fibre) using the Englyst and simulated-GI methods

		Duration of cooking (min)			
		0	10	20	30
A. Englyst	Barley	37	39	37	40
	Oatmeal	42	43	46	47*
	Cauliflower	56	55	57	58
	Carrot	49	53	52	50
B. GI	Barley	23	26*^{ab}	32**^{,a}	35***^{,b}
	Oatmeal	28	34*^{,a}	38**	40***^{,a}
	Cauliflower	34	37^{a}	39*	42**^{,a}
	Carrot	32	35^{a,b}	42**^{,a}	47***^{,b}

Values differ significantly from first row value (no cooking): *$p \leq 0{\cdot}05$, ** $p \leq 0{\cdot}01$,***$p \leq 0{\cdot}001$

^{a,b} Row values with the same subscript also differ significantly ($p \leq 0{\cdot}05$)

1988) are of doubtful value to the nutritionist, who more often desires a knowledge of food components as consumed and active in the gut.

The results in Table 3 are based on the amount of water-soluble fibre in the food samples after exposure to the Englyst, simulated-GI, and in-vivo gastric conditions, respectively. The in-vivo figures do not show the fibre that was actually free in the gastric lumen at the time of sampling but, rather, the effect of the in vivo conditions on water solubility. The diets were a mixture of the dried sample and ground rat pellets, so that the figures are not directly comparable with those in other tables. With all diets, cereal- and vegetable-based, the amounts of fibre made soluble using the simulated gastric and in-vivo gastric conditions, respectively, were similar, but lower than those solubilized using the Englyst procedure. The latter therefore gave a less-valid measure of fibre solubility *in vivo* in the upper monogastric gut.

The above results have shown that the use of non-physiological (phosphate) buffer combined with high temperature, as used in both the Englyst and Prosky

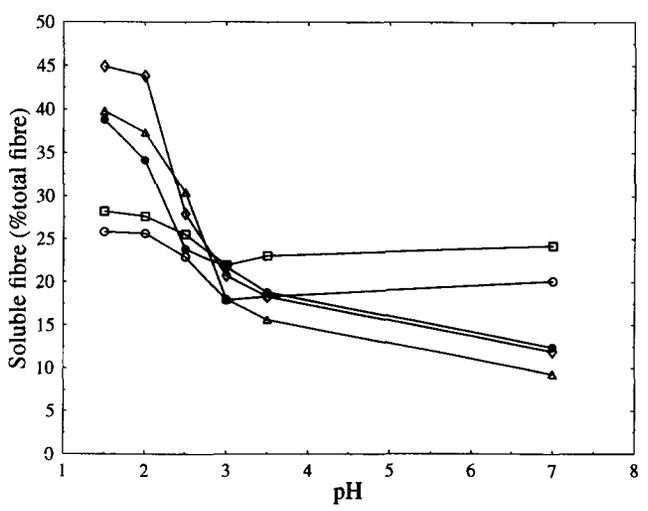

Fig. 4. Effect of pH on fibre extraction by gastric medium with pH set as above (Fig. 3). Barley (○), oatmeal (□), cauliflower (●), carrot (Δ), orange (◇).

Table 3. Soluble fibre extraction (% total fibre): Englyst method, GI conditions and *in vivo* (rat)

	Extraction method		
Feed component^{a}	Englyst^{b}	Simulated-GI^{a}	*in vivo*^{c}
Barley	30·5	23·8	23·3
Oatbran	36·2	24·1	22·4
Cauliflower	43·6	23·1	25·3
Celery	41·4	31·8	25·6
Carrot	46·8	23·6	17·0

^{a} Powdered samples mixed with ground rat pellets to provide diets containing unextracted soluble fibre for comparison of conditions.

^{b,c} All values in columns with differing superscripts differed significantly between columns ($p < 0{\cdot}05$).

methods, leads to an over-estimation of the soluble fibre that would be released *in vivo* from a food. But they also show that nutritional relevance in soluble fibre analysis must be attended by tight control over analytical conditions. To satisfy the criteria for usefulness of scientific data (Reynolds, 1971), namely (a) that there be agreement within the scientific community about the meaning of the terms used and (b) that the results of research contribute systematically to a body of scientific knowledge (including nutrition in the present context), it is imperative that the scientific community adopts a single set of physiological conditions for measuring soluble fibre, and adheres strictly to them. Then, the present problems of lack of relevance and standardization may be jointly solved.

The solubility of dietary fibre *in vivo* will in reality be dependent upon a complex of factors within the food and the GI environment, such as food particle size, various food constituents, meal volume, individual differences, and so on, as others have pointed out (Marlett, 1990; Schweizer, 1990). There is clearly some need for compromise. It is therefore proposed that finely ground samples be used so that the measurement (and thus the definition) of soluble fibre becomes dependent solely upon the chemical integrity of the fibre complex within the average GI environment, as this is the lowest common denominator across a range of situations. A measurement of soluble fibre reduced to dependence upon chemical bonding within the cell-wall matrix, and solubility in the chemical environment of the GI tract, is the most practical option for the production of generalizable analytical data.

The essential features of the simulated GI procedure, compared with the current AOAC and Englyst methods, are summarized in Table 4.

The research described here has been aimed towards a valid measurement of fibre that is soluble in the upper GI tract, by a method compatible with the defining feature of dietary fibre — that it is 'resistant to hydrolysis by the digestive enzymes of man' (Trowell *et al.*, 1976). But it qualifies this definition by adding '...under the conditions in which these enzymes would normally act'.

As long as soluble fibre values are being used in a bi-

Table 4. Characteristics of current fibre methods and simulated-GI method for soluble-fibre measurement

	AOAC/Englyst	Simulated-GI
Soluble-fibre extraction	By conditions of starch digestion + hot phosphate buffer	By sample digestion with HCl/NaCl—pepsin, pH 2·0, 2 h, Tyrode buffer/pancreatin pH 7·0, 2 h
Sequence of steps	**Starch digestion /soluble-fibre extraction** Insoluble fibre 80% EtOH Soluble fibre Starch digest-soluble sugars	**Soluble-fibre extraction** Insoluble fibre and starch 80% EtOH Soluble fibre Soluble sugars **Starch digestion** 80% EtOH Insoluble fibre Starch digest
Soluble-fibre measurement	By difference between fibre values with and without recovery of fibre soluble in starch digest, or gravimetrically	By difference between total NSP values with and without simulated-GI digestion, or by recovery of soluble fibre
Effective definition of soluble fibre	NSP soluble under conditions chosen for starch digestion	NSP soluble under GI conditions
Priority	Elimination of starch	Nutritional relevance
Choice of conditions	Arbitrary—not linked to physiology	Determined by, and precisely standardised to, conditions of average human GI tract
Sensitivity to food processing	Largely insensitive to effects of cooking	Sensitive to effects of cooking

ological/nutritional context the methods used to obtain them should be aligned with the systems in which the fibre may be functional. Therefore, if soluble-fibre measurement is to be related to health and physiology we have little choice but to adhere to the conditions set by the human body, and to work around these to eliminate starch, as a necessary but secondary step in the analytical process.

CONCLUSIONS

1. The nutritional validity of soluble-fibre measurements using current methods is questionable.
2. Priority should be given to nutritionally valid measurement of soluble fibre rather than to starch digestion.
3. Conditions should be dictated by the gut environment and not by analysts.
4. Conditions chosen must be strictly standardized.

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