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Sources of error in dietary fibre analysis

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The following error sources in the present dietary fibre (DF) analytical methods were investigated: (1) the omission of the protease treatment of samples may modify the results by increasing the Klason lignin fraction and altering the content and/or distribution of polysaccharides; (2) some soluble dietary fibre (SDF) constituents can be retained in the insoluble dietary fibre (IDF) matrix affecting the insoluble and soluble fraction distribution; (3) protein, ash and blank corrections in gravimetric analysis involve a lack of precision, over- or undervaluing the actual DF contents; (4) the Klason lignin fractions obtained by acid hydrolysis of DF residues are made up of different components and artifacts besides lignin.

These studies included both new observations and additional quantitative evidence on error sources previously mentioned in the literature. In some cases the published methods were modified to emphasize the methodological errors.

INTRODUCTION

Methods of analysing dietary fibre (DF) have undergone extensive development in the last two decades.

At present, the most widely used methods are the enzymatic–gravimetric AOAC official method (Prosky *et al.*, 1988) and the enzymatic–chemical Englyst method (Englyst & Cummings, 1988); the first is the legal or recommended procedure in at least ten countries, including the USA (Schweizer, 1989) and the second has been recommended as the official method in the United Kingdom (Englyst & Cummings, 1988).

New modifications of the AOAC method are continuously being proposed: Li and Andrews (1988) simplified the method by using a single enzymatic treatment (amyloglucosidase) to determine TDF; Jeraci *et al.* (1989, 1990) used urea enzymatic dialysis to determine TDF or IDF and SDF, and they obtain a lower crude protein and ash contamination of the residues than the AOAC method; Lee and Hicks (1990) replaced the phosphate buffer with MES–TRIS buffer to improve the precision of the assay; Mañas *et al.* (1990) used a single enzymatic treatment (protease) for the determination of IDF and SDF in citrus samples; Li and Cardozo (1992) proposed a non-enzymatic–gravimetric method for the determination of TDF in fruits and vegetables; Prosky *et al.* (1992) recommended simultaneous use of the methods for TDF and IDF determination (Prosky *et al.*, 1988) and that the SDF value be obtained as the difference between these two values, because the AOAC method for quantifying SDF was not precise.

Other methodologies have been proposed by different authors and are also included among the enzymatic–gravimetric methods: Arrigoni *et al.* (1984) used a three step enzymatic treatment that obtains SDF by dialysis and freeze-drying; Mongeau and Brassard (1986) used a rapid enzyme–NDF (neutral detergent fibre) procedure supplemented with a separate procedure for SDF; Brillouet *et al.* (1988) quantified the IDF residue gravimetrically and the SDF fraction chemically.

With regard to the enzymatic–chemical methodologies, the present Englyst method (Englyst & Cummings, 1988) is the result of different reviews (Englyst & Cummings, 1984; Englyst & Hudson, 1987) of the procedure published in 1982 (Englyst *et al.*, 1982), which in turn was a modification of the Southgate method (Southgate, 1969, 1981). The Englyst procedure quantifies DF as non-starch polysaccharides as the best index of plant cell wall material in foods, and ignores lignin determination.

Other enzymatic–chemical approaches to DF are those of Schweizer and Wursch (1979), Jeltema and Zabik (1980), Selvendran and Du Pont (1980), Faulks and Timms (1985), Theander and Westerlund (1986), Anderson and Bridges (1988), Brillouet *et al.* (1988)

(the official method in Australia, Marlett, 1988). All follow different enzymatic and chemical steps to measure DF as neutral plus acidic sugars, and most determine the lignin fraction as Klason lignin.

Although different analytical procedures have been extensively compared (Cummings *et al.*, 1985; Dysseler & Jacqmain, 1985; Marlett & Chesters, 1985; Mongeau & Brassard, 1986; Saura-Calixto, 1987; Schinagel & Tovar, 1987; Marlett & Navis, 1988; Goñi *et al.*, 1989; Marlett *et al.*, 1989; Mongeau & Brassard, 1990; Ravindran & Palmer, 1990; Guillén *et al.*, 1991; Lohmann *et al.*, 1991; Wolters *et al.*, 1992), few papers have addressed the possible error sources (connected with the methodology) in the different procedures.

We have recently reported some errors relating to the gravimetric quantification of DF in citrus samples (Mañas *et al.*, 1990) and the ethanolic precipitation of SDF (Mañas & Saura-Calixto, 1993).

The present paper attempts to study other possible error sources that can be associated with the present DF analytical methods. Experiments were conducted to study the four specific sources listed below.

Omission of the protease treatment

Most of the proposed enzymatic–gravimetric methods carry out a protein hydrolysis step. Some authors do not consider this necessary, since residual protein is always corrected for at the end of the analytical process.

Generally, the enzymatic–chemical methods do not hydrolyse the sample protein, because the DF value is obtained as polysaccharides plus lignin.

However, protein binds strongly to DF components as well as to other dietary constituents (Stevens & Selvendran, 1984*a,b,c*; O'Neill & Selvendran, 1985; Redgwell & Selvendran, 1986). This fact might result in different fraction distributions and/or a total DF content, depending upon whether or not the protein was present during the analysis.

Therefore, the effect of the presence of protein during DF fraction analysis was studied.

SDF quantification

Problems associated with the ethanolic precipitation of SDF have recently been reported (Mañas & Saura-Calixto, 1993). Both non-fibre component co-precipitation and incomplete precipitation of SDF components were observed. Dialysis was considered as an alternative method, and more reliable results were obtained.

Some physiological effects of DF are closely related to the water solubility of its components. Therefore, obtaining individual values for IDF and SDF gives important information about the physiological properties of the DF.

The present DF analytical methods consider SDF to be the fibre fraction solubilized at 100°C in either pH 6·0 buffer (Prosky *et al.*, 1988) or pH 7·0 buffer (Englyst & Cummings, 1988).

The physical and chemical separation conditions (pH, temperature, concentration, buffer, etc.) might affect and vary the distribution of the DF fractions. Moreover, IDF constituents form a matrix which can retain other substances from the analytical solutions. Some of these compounds might be constituents of the SDF fraction, which would then be quantified as IDF and not SDF.

Soluble DF constituent retention in the IDF matrix was studied by modifying the conditions of fraction separation.

Ash and protein correction in DF residues

Some components can be retained in the residues or precipitated along with the DF constituents when enzymatic–gravimetric methods are used.

The corrections performed by the current methods are referred to protein, blank and ash, which may involve quantitative errors.

The precision of the gravimetric analysis was determined by comparing the results obtained by gravimetric and by chemical methods.

Composition of the Klason lignin fraction

The enzymatic–chemical methods quantify lignin as the residue obtained after acid hydrolysis of the IDF fraction.

Different authors have reported that this residue is not lignin alone; it also contains other acid-insoluble components, such as cutins, polyphenols, protein condensed products, etc. (Van Soest & Wine, 1968; Heredia, 1979; Jeltema & Zabik, 1980; Southgate, 1981; Theander & Aman, 1982).

To study the possible contribution of other components present in the IDF residue to the KL fraction, acid hydrolysis was performed in some synthetic standard mixtures.

MATERIAL & METHODS

Samples

Fruits, legumes and cereals were selected as test pieces in order to study samples with clearly different DF contents and composition. They are also common in the human diet and used as ingredients of DF-enriched products.

Apple and citrus (orange pulp and peel, lemon pulp) samples were wastes obtained after industrial juice extraction. Citrus samples were washed with 96% ethanol to eliminate pigments, free sugars and organic acids.

Bean and oat-flake samples were obtained at a local supermarket. Oat flakes were previously defatted to a content of less than 5% fat, as recommended in the DF analytical methodologies.

All samples were milled to a particle size of less than 0·5 mm, and moisture content was determined by drying to constant weight at 105°C.

Standards were: lignin (Eucalin Kraft Lignin, ENCESA, Spain), cellulose (Sigmacell Cellulose Type 100, SIGMA Chemicals, St. Louis, MO, USA), casein (Hammarsten Casein, MERCK, Darmstadt, Germany) and pectin (citrus fruit pectins, SIGMA).

Enzyme preparations (heat-stable α-amylase A-3306, protease P-3910 and amyloglucosidase A-9913) were purchased from SIGMA.

All chemicals were of analytical grade.

Dialysis equipment

A continuous water-renovation system was used. It consisted of running water that was preheated by passing through a 20-m length latex tube immersed in a 40°C water-bath. The preheated water was propelled with a peristaltic pump to the bottom of a 43 litre dialysis chamber made of methacrylate kept at 25°C with a thermostat. Water flow was 7 litres/h, which implies four reservoir volumes a day.

GLC apparatus

An HP-5890 A (Hewlett-Packard, Avondale, PA, USA) chromatograph fitted with a flame ionization detector and autoinjector, and connected to an HP-3390 A computing integrator, was used. The column was an SP-2330 capillary column, 30 m × 0·32 mm i.d. (Supelco Cat. No. 2-4073) (Supelco, Bellefonte, PA, USA). Operating conditions were: column temperature 240°C (isothermal); injector temperature 270°C; detector temperature 250°C; carrier gas (nitrogen) flow rate 3 ml/min.

Spectrophotometer

The spectrophotometer was a double beam Lambda 2 (UV/VIS) (Perkin-Elmer, Norwalk, CT, USA).

Nitrogen determination

For this, a 1030 Kjeltec Autoanalyzer (Tecator, Höganös, Sweden) was used.

Analysis

At least three replications of all the treatments were performed for each analysed product.

Study of the protease treatment (Scheme in Fig. 1)

Samples: orange pulp, beans, oat flakes.

Preparation of fibre fractions

DF fractions were obtained by a modification of the AOAC method developed in our laboratory. Reagents and conditions were the same as in the AOAC method (Prosky *et al.*, 1988), but only half of the sample and reagents required in the AOAC method were needed because the entire experiment was carried out in a 50 ml screw-cap glass centrifuge tube. IDF was obtained by centrifugation (1000 *g*, 15 min) of the sample, rather than by filtration, collecting the resulting supernatant and washes with a Pasteur pipette. Dialysis replaced ethanol precipitation to obtain SDF. These modifications were performed to avoid several previously reported error sources (Mañas *et al.*, 1990; Mañas & Saura-Calixto, 1993).

In these experiments, enzymatic treatments were carried out either following the entire AOAC method or omitting the protease treatment. Heat-stable α-amylase and amyloglucosidase treatments were always omitted in citrus samples because they do not contain starch.

IDF determination

IDF residues obtained after enzymatic treatment and centrifugation were hydrolysed under previously tested conditions which included sequential 12 M (1 h, 30°C) and 1 M (1·5 h, 100°C) sulphuric acid hydrolysis steps.

Neutral sugars (NS) and uronic acids (UA) were quantified in the hydrolysates: NS by GLC as alditol acetates (Englyst & Cummings, 1988), and UA by spectrophotometry (Scott, 1979). The residues were dried (105°C, constant weight) and quantified as Klason lignin (KL).

IDF was calculated as

$$IDF = NS + UA + KL$$

SDF determination

The combined supernatant liquid and water washings, obtained from the centrifugation step for IDF residues, were dialysed. Dialysis was carried out for 48 h against water using a 12 000–14 000 MW cut-off dialysis bag (Dialysis Tubing Visking 9-32/36 mm, Medicell International, Ltd.) and the dialysis system described above.

Dialysed samples were hydrolysed in 1 M sulphuric acid (1·5 h, 100°C), and NS and UA were quantified in the hydrolysates following the same techniques as in IDF determination.

SDF was calculated as

$$SDF = NS + UA$$

Protein determination

Total nitrogen was determined in the KL residues by the Kjeldahl method. Protein was calculated as $N \times 6{\cdot}25$.

Retention of soluble DF constituents in the IDF matrix (Scheme in Fig. 2)

Samples: orange pulp and peel, lemon pulp, apple, beans, oat flakes.

The unmodified AOAC method (Prosky *et al.*, 1988) was followed to obtain IDF residues. Some IDF residues were gravimetrically quantified (IDFr).

In parallel, other IDF residues were treated with distilled water (40 ml, 100°C, 30 min, twice), and super-

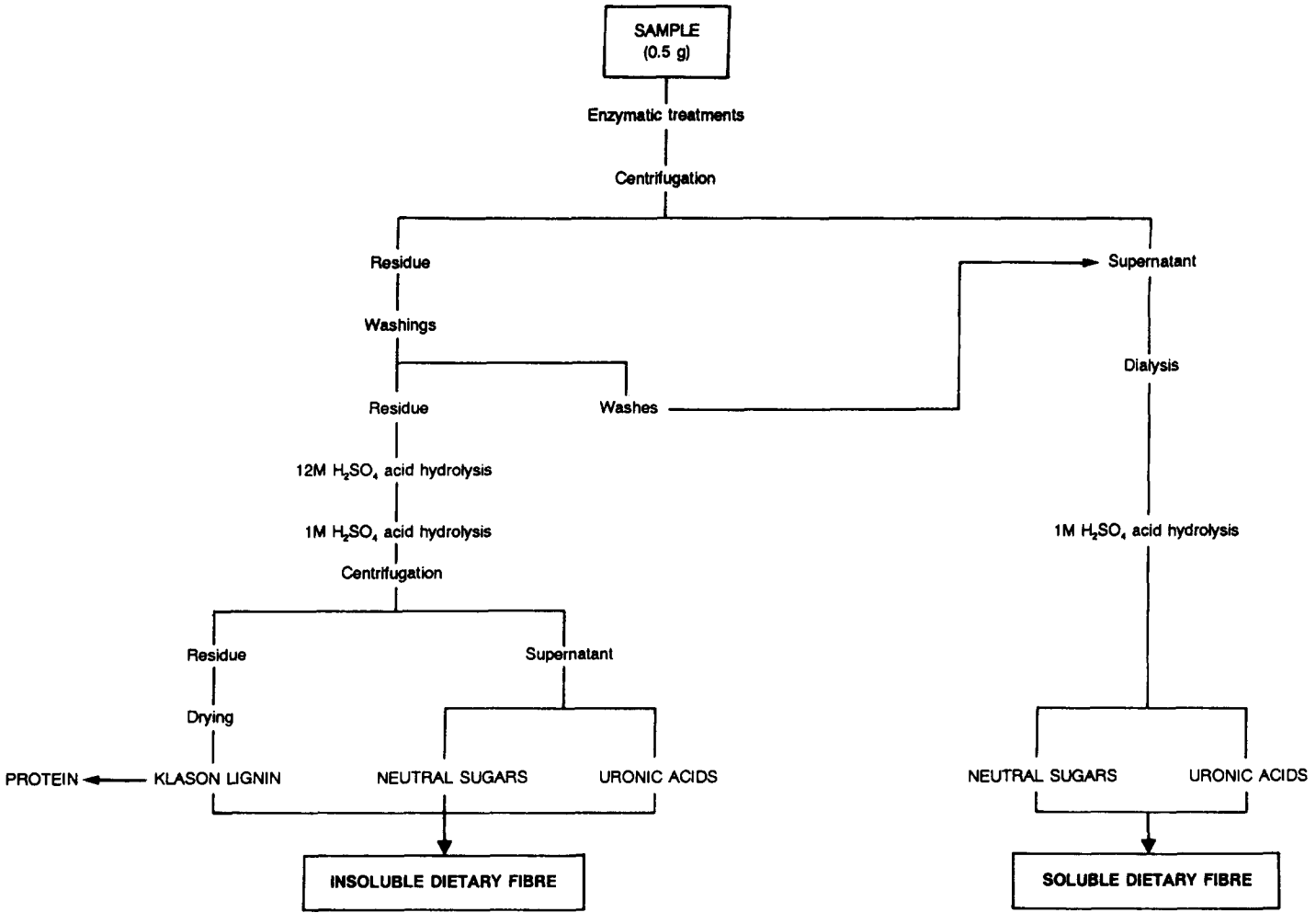

Fig. 1. Scheme for protease treatment study.

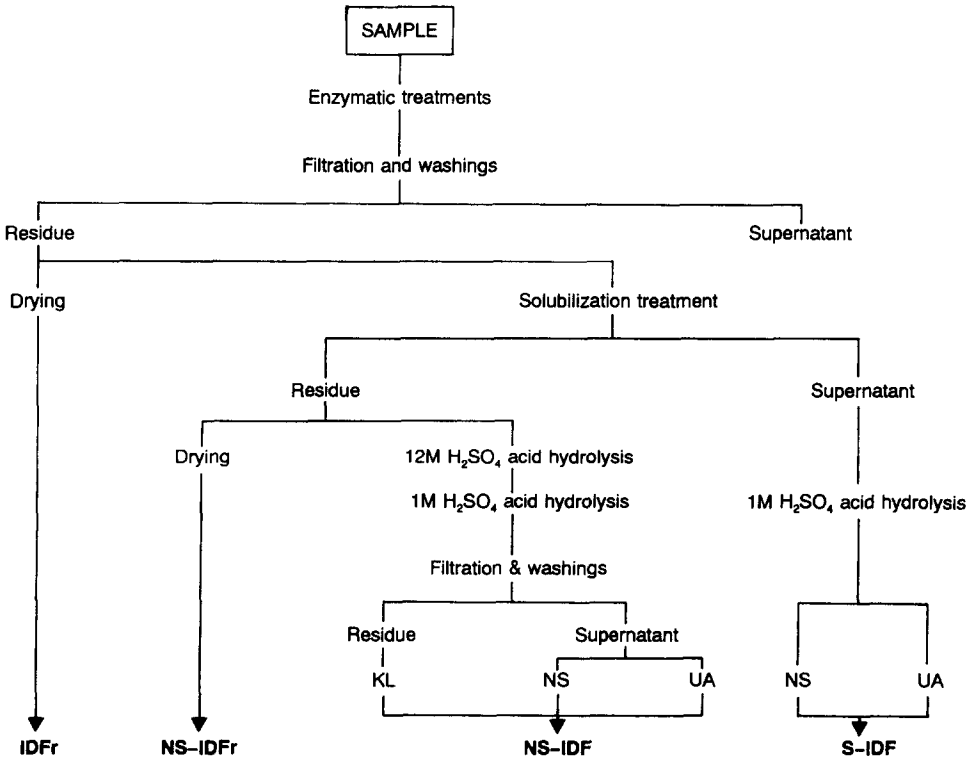

Fig. 2. Scheme for retention of SDF constituents in the IDF matrix study.

natants collected. IDF was quantified in non-solubilized residues as indicated in the AOAC method (NS-IDFr), or else hydrolysed and quantified chemically (NS-IDF) following the above-described procedure. The NS and UA contents were also determined in supernatants (S-IDF).

Ash and protein correction in DF residues

Samples: orange pulp and peel, lemon pulp, apple, beans, oat flakes.

The unmodified AOAC method (Prosky *et al.*, 1988) was followed to quantify IDF and SDF fractions.

Some IDF and SDF residues obtained by the AOAC method were also hydrolysed and analysed as described above.

Acid hydrolysis of standards (Klason lignin analysis)

Standards: lignin, cellulose, casein, pectins.

The above mentioned products, as well as some of their binary or ternary mixtures, were hydrolysed in 12 M and 1 M sulphuric acid under the conditions previously described. Hydrolysis residues were washed with distilled water, dried (105°C, constant weight) and gravimetrically quantified.

RESULTS AND DISCUSSION

Protease treatment

Tables 1, 2 and 3 show the results of the IDF and SDF determinations carried out on orange pulp, beans and oat flakes, respectively, with or without the protease treatment.

The protease treatment affected the non-starch polysaccharide (NSP) content in different ways depending upon the type of sample. Orange samples showed a higher NSP content in the SDF fraction when the protease treatment was performed, while no significant differences were observed in the IDF fractions. Bean samples showed a significantly different insoluble and soluble NSP distribution depending upon the presence or absence of protein: NSP were lower in the IDF fraction and higher in the SDF fraction when the protease treatment was performed than when it was omitted. Oat samples showed significant differences in the IDF, but not in the SDF, fractions.

With regard to the KL fractions, it can be observed that these fractions, as well as their protein contents, were always higher when the protease treatment was omitted. This is in agreement with the known strong association between lignin and protein. The greatest differences were found in bean samples, and could be explained on the basis of a lower soluble protein content of beans as compared with the other samples.

Nevertheless, the higher KL value is not only accounted for by the higher protein content but also by the presence of other substances, such as polysaccharides and polyphenols. These compounds may condense with lignin and protein (Heredia, 1979; Theander & Aman, 1982) and so remain in the KL fractions.

The results suggest that the presence of protein during DF analysis directly affects the KL fraction and may also affect NSP content and distribution.

Since proteolytic enzymes are present in the human gastrointestinal tract, protein hydrolysis should be done during DF analysis, in order to obtain in vitro results that were closer to the in vivo results.

Table 1. Orange pulp IDF and SDF contents obtained with or without protease treatment (% dry matter)

	With protease		Without protease	
	IDF	SDF	IDF	SDF
Rha	0·96 ± 0·06	0·18 ± 0·02	0·83 ± 0·03	0·13 ± 0·02
Fuc	0·39 ± 0·12	0·06 ± 0·02	0·41 ± 0·10	0·05 ± 0·01
Ara	6·00 ± 0·34	2·73 ± 0·06	5·35 ± 0·39	2·54 ± 0·21
Xyl	1·97 ± 0·09	0·08 ± 0·01	1·79 ± 0·06	0·06 ± 0·01
Man	1·45 ± 0·05	0·20 ± 0·02	1·37 ± 0·03	0·18 ± 0·02
Gal	5·29 ± 0·15	2·02 ± 0·05	5·09 ± 0·05	1·89 ± 0·16
Glu	13·31 ± 0·27	0·39 ± 0·02	12·68 ± 0·32	0·36 ± 0·03
ΣNS[1]	29·37 ± 0·49	5·66 ± 0·09	27·52 ± 0·52	5·24 ± 0·27
UA[2]	16·27 ± 0·31	7·94 ± 0·29	14·00 ± 1·24	7·96 ± 0·67
NSP	45·64 ± 0·58	13·60 ± 0·30[a]	41·52 ± 1·34	13·20 ± 0·72[a']
KL	7·40 ± 0·47		9·21 ± 1·14	
KLP[3]	0·68 ± 0·13		1·46 ± 0·27	
NSP+KL	53·04 ± 0·75		50·73 ± 1·76	
TDF	66·64 ± 0·81		63·93 ± 1·90	

Mean value ± standard deviation ($n \geq 3$).
a–a' were significantly different: $P < 0{\cdot}01$.
[1] NS: neutral sugars.
[2] UA: uronic acids.
[3] KLP: Klason lignin protein.

Table 2. Bean IDF and SDF contents obtained with or without protease treatment (% dry matter)

	With protease		Without protease	
	IDF	SDF	IDF	SDF
Rha	0·06 ± 0·01	0·07 ± 0·01	0·06 ± 0·01	0·05 ± 0·01
Fuc	t[1]	0·20 ± 0·01	0·12 ± 0·01	0·18 ± 0·01
Ara	1·58 ± 0·13	1·25 ± 0·04	1·93 ± 0·13	1·05 ± 0·07
Xyl	0·34 ± 0·02	0·48 ± 0·02	0·38 ± 0·01	0·42 ± 0·02
Man	t[1]	0·76 ± 0·05	0·55 ± 0·02	0·33 ± 0·02
Gal	0·54 ± 0·06	0·64 ± 0·02	0·67 ± 0·05	0·52 ± 0·04
Glu	6·21 ± 0·12	0·56 ± 0·02	6·11 ± 0·28	0·51 ± 0·03
ΣNS[2]	8·73 ± 0·19	3·96 ± 0·07	9·82 ± 0·31	3·06 ± 0·09
UA[3]	1·81 ± 0·17	0·94 ± 0·08	2·45 ± 0·19	0·47 ± 0·07
NSP	10·54 ± 0·25[a]	4·90 ± 0·11[b]	12·27 ± 0·36[a']	3·53 ± 0·11[b']
KL	2·38 ± 0·03[c]		6·71 ± 0·41[c']	
KLP[4]	0·81 ± 0·10		4·03 ± 0·40	
NSP+KL	12·92 ± 0·25		18·98 ± 0·54	
TDF	17·82 ± 0·27		22·51 ± 0·55	

Mean value ± standard deviation ($n \geq 3$).
a–a', *b–b'* and *c–c'* were significantly different : $P < 0{\cdot}01$.
[1] t: trace.
[2] NS: neutral sugars.
[3] UA: uronic acids.
[4] KLP: Klason lignin protein.

SDF quantification

As can be observed in Table 4, the IDF residue value obtained after the solubilization treatment (NS-IDFr) was lower than the IDF value obtained with the AOAC method (IDFr), the differences being significant in all the studied samples. Only a part of the solubilized fraction [(IDFr) − (NS-IDFr)] was actually fibre (S-IDF), suggesting that other components, initially retained in the IDF matrix, were also solubilized.

On the other hand, when the NS-IDFr fraction was chemically quantified, the resulting value (NS-IDF) was slightly lower than the gravimetric one. These differences are not statistically significant, and are minimized when the protein and ash corrections are performed on the NS-IDFr residue.

As previously mentioned, the present DF analytical methods consider SDF as the DF fraction solubilized at 100°C. On this basis, polysaccharides solubilized when IDF was treated in boiling water would also be considered SDF.

Table 3. Oat-flake IDF and SDF contents obtained with or without protease treatment (% dry matter)

	With protease		Without protease	
	IDF	SDF	IDF	SDF
Rha	0·01 ± 0·01	0·02 ± 0·01	0·02 ± 0·01	0·02 ± 0·01
Fuc	t[1]	0·01 ± 0·00	t[1]	0·01 ± 0·00
Ara	0·67 ± 0·02	0·20 ± 0·02	0·70 ± 0·01	0·17 ± 0·01
Xyl	0·95 ± 0·04	0·14 ± 0·01	1·02 ± 0·01	0·13 ± 0·00
Man	0·12 ± 0·01	0·17 ± 0·01	0·14 ± 0·01	0·14 ± 0·01
Gal	0·09 ± 0·01	0·24 ± 0·01	0·11 ± 0·01	0·18 ± 0·01
Glu	1·04 ± 0·03	2·26 ± 0·06	1·08 ± 0·01	2·31 ± 0·02
ΣNS[2]	2·88 ± 0·06	3·04 ± 0·07	3·07 ± 0·02	2·96 ± 0·03
UA[3]	0·33 ± 0·01	0·21 ± 0·01	0·35 ± 0·01	0·26 ± 0·01
NSP	3·21 ± 0·06[a]	3·25 ± 0·07	3·42 ± 0·02[a']	3·22 ± 0·03
KL	2·22 ± 0·92[b]		3·51 ± 0·50[b']	
KLP[4]	0·49 ± 0·10		1·71 ± 0·26	
NSP+KL	5·43 ± 0·92		6·93 ± 0·50	
TDF	8·68 ± 0·92		10·15 ± 0·50	

Mean value ± standard deviation ($n \geq 3$).
a–a' were significantly different: $P < 0{\cdot}01$.
b–b' were significantly different: $P < 0{\cdot}05$.
[1] t: trace.
[2] NS: neutral sugars.
[3] AU: uronic acids.
[4] KLP: Klason lignin protein.

Table 4. Effect of the solubilization treatment performed in IDF residues (% dry matter)

	Orange pulp	Orange peel	Lemon pulp	Apple pulp	Beans	Oat
IDFr[1]	62·59 ± 0·46^{a}	60·07 ± 1·15^{b}	44·44 ± 0·32^{c}	29·70 ± 0·28^{d}	17·82 ± 0·11^{e}	7·21 ± 0·27^{f}
NS-IDFr[2]	41·76 ± 0·62$^{a'}$	39·64 ± 2·70$^{b'}$	27·21 ± 0·89$^{c'}$	21·34 ± 0·98$^{d'}$	10·75 ± 0·61$^{e'}$	5·04 ± 0·42$^{f'}$
NS-IDF[3]	39·38 ± 0·53	36·29 ± 0·93	25·72 ± 0·78	21·54 ± 0·29	10·86 ± 0·21	4·78 ± 0·25
S-IDF[4]	7·12 ± 0·21	13·03 ± 0·44	8·13 ± 0·49	3·20 ± 0·08	4·99 ± 0·07	1·25 ± 0·07

Mean value ± standard deviation ($n > 3$).
a–a', b–b', c–c' and e–e' were significantly different: $P < 0·001$.
d–d' and f–f' were significantly different: $P < 0·01$.
[1] IDFr: IDF residue.
[2] NS-IDFr: non-solubilized IDF residue.
[3] NS-IDF: non-solubilized IDF = neutral sugars + uronic acids + Klason lignin.
[4] S-IDF: solubilized IDF = neutral sugars + uronic acids.

Tables 5 and 6 show the results obtained with NS-IDFr and S-IDF chemical analysis. Among neutral sugars (NS), glucose was the major monosaccharide in NS-IDFr, as would be expected in an IDF fraction. Arabinose and galactose were the main monosaccharide, in the S-IDF fraction. These are the commonest neutral sugars in the majority of soluble components of DF (pectins). Additionally, the high uronic acid (UA) content found in the S-IDF fraction suggests that it may be considered SDF.

Analytical separations of IDF and SDF are just a simple approximation of what really takes place in the organism. The closer the physiological conditions are to the analytical ones, the closer the approximation is expected to be. Nevertheless, it is essential to establish a clear, uniform concept of SDF. If the idea of chemical solubility is maintained, the solubilization treatment at 100°C should be used to obtain greater accuracy in the IDF and SDF values. Another option would be to carry out this solubilization treatment at 37°C, which is a more physiological temperature, to get closer results to the situation *in vivo*.

Ash and protein correction in DF residues

Table 7 shows the results obtained with the gravimetric DF analysis (AOAC method, Prosky *et al.*, 1988) of the studied samples. Table 8 indicates the percentages that each of the corrections performed represents over the total amount of the gravimetric residues.

Although the values obtained for protein content in the gravimetric residues in the different samples were quite similar (Table 7), their relative values with regard to the total residue were quite different and ranged from 1·5% in the lemon-pulp SDF to 32·3% in the oat flake IDF (Table 8). The AOAC method, like most of the enzymatic—gravimetric methods, quantifies protein quantity by analysing the amount of nitrogen in the residues, and then using 6·25 as a conversion factor. This method assumes that all the nitrogen in the sample is in proteins, and that all proteins have the same nitrogen : protein ratio. It must not be forgotten that the nitrogen : protein ratio depends upon the substance being analysed, and ranges from 5·18 in almonds to 6·31 in wheat bran (Holland *et al.*, 1991).

With regard to ash content, the SDF fractions in the citric samples showed the highest values. Enzymatic treatments of citric samples finished at pH 7·5, because the starch hydrolysis steps were omitted, and this could explain the high ash values observed. Co-precipitation of salts and/or acids along with the SDF constituents is greater at pH 7·5 than at pH 4·5, which is the pH at which enzymatic treatments of starchy foods finish.

Table 5. Chemical composition of the fractions obtained with the solubilization treatment of the citrus IDF residues (% dry matter)

	Orange pulp		Orange peel		Lemon pulp	
	NS-IDF[1]	S-IDF[2]	NS-IDF[1]	S-IDF[2]	NS-IDF[1]	S-IDF[2]
Rha	0·35 ± 0·02	0·51 ± 0·07	0·43 ± 0·02	0·36 ± 0·08	0·35 ± 0·03	0·55 ± 0·10
Fuc	0·18 ± 0·02	0·05 ± 0·02	0·21 ± 0·03	0·05 ± 0·02	0·30 ± 0·03	0·10 ± 0·02
Ara	3·67 ± 0·15	1·82 ± 0·20	2·96 ± 0·33	2·04 ± 0·14	0·93 ± 0·11	1·11 ± 0·05
Xyl	1·47 ± 0·02	0·08 ± 0·02	1·33 ± 0·15	0·11 ± 0·02	1·94 ± 0·11	0·34 ± 0·02
Man	1·25 ± 0·05	t[3]	1·20 ± 0·13	0·25 ± 0·02	1·57 ± 0·06	0·13 ± 0·05
Gal	3·29 ± 0·12	1·71 ± 0·17	2·73 ± 0·28	2·10 ± 0·21	1·43 ± 0·13	0·88 ± 0·05
Glu	12·60 ± 0·15	0·40 ± 0·03	12·32 ± 0·46	0·36 ± 0·06	15·46 ± 0·34	0·63 ± 0·05
ΣNS[4]	22·81 ± 0·25	4·57 ± 0·27	21·18 ± 0·66	5·27 ± 0·27	21·98 ± 0·40	3·74 ± 0·14
UA[5]	11·18 ± 0·51	2·55 ± 0·07	6·89 ± 0·17	7·76 ± 0·39	1·38 ± 0·05	4·39 ± 0·48
NSP	33·99 ± 0·57	7·12 ± 0·28	28·07 ± 0·68	13·03 ± 0·47	23·36 ± 0·40	8·13 ± 0·50
KL	5·39 ± 0·04		8·22 ± 0·79		2·36 ± 0·70	
NSP ± KL	39·38 ± 0·57		36·29 ± 1·04		25·72 ± 0·81	

Mean value ± standard deviation ($n \geq 3$).
[1] NS-IDF: non-solubilized IDF; [2] S-IDF: solubilized IDF.
[3] t: trace.
[4] NS: neutral sugars; [5] UA: uronic acids.

Table 6. Chemical composition of the fractions obtained with the solubilization treatment of apple pulp, beans and oat-flake IDF residues (% dry matter)

	Apple pulp		Beans		Oat flakes	
	NS-IDF[1]	S-IDF[2]	NS-IDF[1]	S-IDF[2]	NS-IDF[1]	S-IDF[2]
Rha	0·08 ± 0·01	0·06 ± 0·01	0·03 ± 0·01	0·02 ± 0·01	0·05 ± 0·01	0·07 ± 0·01
Fuc	0·32 ± 0·01	0·02 ± 0·01	0·04 ± 0·01	0·04 ± 0·00	0·02 ± 0·01	0·02 ± 0·01
Ara	1·10 ± 0·13	0·79 ± 0·02	1·49 ± 0·07	2·63 ± 0·01	0·80 ± 0·04	0·34 ± 0·04
Xyl	1·78 ± 0·14	0·21 ± 0·01	0·76 ± 0·04	0·30 ± 0·01	1·13 ± 0·06	0·37 ± 0·07
Man	0·78 ± 0·05	0·03 ± 0·01	0·05 ± 0·01	0·03 ± 0·01	0·16 ± 0·01	0·02 ± 0·01
Gal	2·07 ± 0·17	0·96 ± 0·02	0·25 ± 0·01	0·48 ± 0·01	0·11 ± 0·01	0·05 ± 0·01
Glu	11·72 ± 0·24	0·11 ± 0·02	4·24 ± 0·24	0·22 ± 0·02	1·30 ± 0·04	0·34 ± 0·06
ΣNS[3]	17·85 ± 0·35	2·18 ± 0·04	6·86 ± 0·25	3·72 ± 0·03	3·57 ± 0·08	1·21 ± 0·10
UA[4]	0·86 ± 0·03	1·02 ± 0·08	1·70 ± 0·05	1·27 ± 0·07	0·22 ± 0·01	0·04 ± 0·01
NSP	18·71 ± 0·35	3·20 ± 0·09	8·56 ± 0·25	4·99 ± 0·08	3·79 ± 0·08	1·25 ± 0·10
KL	2·83 ± 0·17		2·30 ± 0·14		0·99 ± 0·24	
NSP+KL	21·54 ± 0·39		10·86 ± 0·29		4·78 ± 0·25	

Mean value ± standard deviation ($n \geq 3$).
[1]NS-IDF: non-solubilization IDF; [2]S-IDF: solubilized IDF. [3]NS: neutral sugars; [4]UA: uronic acids.

After analysing the blanks, the IDF correction value was smaller than the SDF correction value.

The total corrective value for the DF residues may reach as high as the 88·8% gravimetric residue found in the bean SDF fraction. This indicates that DF can be quantified in a fraction where it is the minority component and this represents a serious error source. On the other hand, it must be remembered that the weight of the celite used as filtration co-adjuvant has already been subtracted from the gravimetric residue value. If we consider the total weight of the residue, including celite, DF would represent a lower fraction of the weight than when this value was calculated from residue weight without celite.

In order to know if the gravimetric quantification of IDF and SDF residues corresponded to actual fibre components, the chemical and gravimetric values of these fractions were compared.

Table 9 shows the chemical analysis results for IDF and SDF fractions obtained by the AOAC method. Gravimetric and chemical values are compared in Table 10.

Gravimetric values were always significantly different from the chemical values, except in the apple pulp SDF fraction.

The gravimetric values were always higher than the chemical values in the fruit samples and in the IDF fraction of the bean samples. The highest differences between the gravimetric and the chemical results were found in the SDF fractions of citrus samples.

Polysaccharides make excellent stationary chromatographic phases, and have the ability to retain many organic and inorganic compounds. On DF analysis, inorganic salts from the buffer, as well as organic and inorganic sample components, may be retained in the IDF matrix and/or precipitate along with the SDF constituents. Both factors, retention and co-precipitation, could explain the overestimated DF value observed in the gravimetric quantification of DF in fruit samples.

As has been previously mentioned, the highest differences were found in the values for the SDF fractions in citrus samples, which would agree with this hypothesis. These fractions consist mainly of pectic substances which give rise to high-viscosity solutions that may favour the retention of different compounds.

On the other hand, the gravimetric values for oat samples and the SDF fraction in bean samples were lower than the chemical ones. This could be explained by incorrect gravimetric quantification of DF because

Table 7. Gravimetric quantification of IDF and SDF of fruits, legumes and cereal samples (% dry matter)

Sample		Residue	Protein	Ash	Blank	$Fibre_G$[1]
Orange pulp	IDF	62·59 ± 0·46	1·82 ± 0·11	6·22 ± 0·50	0·36 ± 0·00	54·19 ± 0·69
	SDF	70·33 ± 1·08	1·17 ± 0·11	51·07 ± 2·87	4·81 ± 0·04	13·28 ± 3·07
Orange peel	IDF	60·07 ± 1·15	2·27 ± 0·40	3·85 ± 0·30	0·35 ± 0·00	53·60 ± 1·25
	SDF	77·19 ± 3·81	1·21 ± 0·11	61·53 ± 2·53	4·66 ± 0·06	9·79 ± 4·58
Lemon pulp	IDF	44·44 ± 0·32	1·07 ± 0·00	1·16 ± 0·29	0·35 ± 0·01	41·86 ± 0·43
	SDF	95·94 ± 0·70	1·50 ± 0·00	57·93 ± 0·56	4·70 ± 0·06	31·81 ± 0·90
Apple pulp	IDF	29·70 ± 0·28	1·85 ± 0·05	0·00	0·31 ± 0·00	27·54 ± 0·28
	SDF	22·36 ± 0·60	2·69 ± 0·05	3·49 ± 0·26	4·01 ± 0·02	12·17 ± 0·66
Beans	IDF	17·82 ± 0·11	1·47 ± 0·03	0·00	0·15 ± 0·00	16·20 ± 0·11
	SDF	7·77 ± 0·38	1·81 ± 0·04	3·15 ± 0·02	1·94 ± 0·01	0·87 ± 0·39
Oat flakes	IDF	7·21 ± 0·27	2·33 ± 0·03	0·00	0·14 ± 0·00	4·74 ± 0·27
	SDF	5·62 ± 0·24	1·42 ± 0·06	1·39 ± 0·05	1·83 ± 0·02	0·98 ± 0·25

Mean value ± standard deviation ($n \geq 3$).
[1]$Fibre_G$ = (residue − protein − ash − blank).

Table 8. Effect of corrections on the IDF and SDF gravimetric quantification (% dry matter)

Sample	Fraction	Residue	Protein	Ash	Blank	Total[1]
Orange pulp	IDF	100	2·9	9·9	0·6	13·4
	SDF	100	1·7	72·0	7·2	80·9
Orange peel	IDF	100	3·8	6·4	0·6	10·8
	SDF	100	1·6	79·7	6·0	87·3
Lemon pulp	IDF	100	2·4	2·6	0·8	5·8
	SDF	100	1·5	60·4	4·9	66·8
Apple pulp	IDF	100	6·2	0·0	1·0	7·2
	SDF	100	12·2	15·6	17·9	45·7
Beans	IDF	100	8·2	0·0	0·8	9·0
	SDF	100	23·3	40·5	25·0	88·8
Oat flakes	IDF	100	32·3	0·0	1·9	34·2
	SDF	100	25·3	24·7	32·6	82·6

[1]Total = protein + ash + blank.

the corrections performed on the residues were actually inaccurate.

All of these results show that the gravimetric quantification of DF was not sufficiently accurate in all of the studied samples and could actually produce an overvalue as large as 166%. Factors such as retention and co-precipitation, as well as propagation of errors caused by the corrections performed in the gravimetric residues, might explain the inaccurate DF values obtained by gravimetric quantification.

Additionally, it must be considered that other components, such as polyphenols and resistant starch, which might be present in the residues,are not taken into account and consequently, if present, they will be quantified as DF (Saura-Calixto *et al.*, 1991, 1993).

Klason lignin fraction composition

Table 11 shows the results obtained in the KL fraction study using the hydrolysis treatment of standard(s).

A hydrolysis residue was found in all of the studied products or their mixtures, as is indicated in the 'KL' headed column, where the weight of the residues is indicated (range of values).

The values of the 'KL–91·36% lignin' column correspond to the cellulose and/or pectins and/or protein residues obtained after subtracting the percentage of lignin present in the sample (91·36% of the initially added lignin) from the KL values corresponding to the mixture of lignin with these products. These results are expressed as a percentage of the initial content of the mentioned products.

As can be observed, cellulose, pectins and protein are not completely hydrolysed by the acid treatment, and a fraction of them remain in the residue, which corresponds to the KL fraction obtained when this treatment is performed as part of DF chemical analysis.

The presence of lignin always produces large amounts of residue, and the residue was greatest when protein accompanied lignin. This indicates that the presence of lignin favours the formation of non-hydrolysable condensed products.

It must be pointed out that, even when lignin is not present, mixtures of cellulose–pectin–protein are not completely hydrolysed, and a residue, which will also be quantified as KL, is produced. This reflects the capacity of protein to form acid-insoluble condensed products in the presence of polysaccharides.

The capacity of both lignin and protein to form condensed products with other compounds may result in incomplete hydrolysis of DF polysaccharide constituents. This non-hydrolysed fraction would also be quantified as KL.

The results using standard mixtures suggest that the

Table 9. Chemical quantification of the IDF and SDF fractions obtained by the AOAC method of citric, cereal and legume samples (% dry matter)

Sample		Neutral sugars	Uronic acids	Klason lignin	Fibre
Orange pulp	IDF	29·64 ± 1·64	19·45 ± 0·91	5·87 ± 0·88	54·96 ± 1·84
	SDF	3·97 ± 0·05	1·58 ± 0·05	—	5·55 ± 0·06
Orange peel	IDF	25·96 ± 0·40	13·63 ± 1·39	11·30 ± 0·11	50·89 ± 1·42
	SDF	3·75 ± 0·12	1·59 ± 0·09	—	5·34 ± 0·12
Lemon pulp	IDF	25·65 ± 0·23	7·28 ± 0·71	4·71 ± 0·95	37·64 ± 1·21
	SDF	4·09 ± 0·08	11·64 ± 0·44	—	15·73 ± 0·44
Apple pulp	IDF	19·65 ± 0·12	2·15 ± 0·02	4·77 ± 0·08	26·57 ± 0·14
	SDF	4·46 ± 0·07	8·27 ± 0·32	—	12·73 ± 0·32
Beans	IDF	10·50 ± 0·18	3·08 ± 0·13	2·38 ± 0·03	15·96 ± 0·22
	SDF	0·98 ± 0·04	0·54 ± 0·03	—	1·52 ± 0·04
Oat flakes	IDF	4·57 ± 0·10	0·28 ± 0·01	2·22 ± 0·92	7·07 ± 0·92
	SDF	3·06 ± 0·17	0·11 ± 0·01	—	3·17 ± 0·17

Mean value ± standard deviation ($n > 3$).

Table 10. Comparison of gravimetric and chemical values obtained on the analysis of AOAC fraction (% dry matter)

Sample	Fraction	Gravimetric (*G*)	Chemical (*Ch*)	(*G*/*Ch*) × 100
Orange pulp	IDF	54·19 ± 0·69	54·96 ± 1·84	108
	SDF	13·28 ± 3·07	5·55 ± 0·06	266
Orange peel	IDF	53·60 ± 1·25	50·89 ± 1·42	114
	SDF	9·79 ± 4·58	5·34 ± 0·12	203
Lemon pulp	IDF	41·86 ± 0·43	37·64 ± 1·21	122
	SDF	31·81 ± 0·90	15·73 ± 0·44	225
Apple pulp	IDF	27·54 ± 0·28	26·57 ± 0·14	113
	SDF	12·17 ± 0·66	12·73 ± 0·32	106
Beans	IDF	16·20 ± 0·11	15·96 ± 0·22	111
	SDF	0·87 ± 0·39	1·52 ± 0·04	63
Oat flakes	IDF	4·74 ± 0·27	7·07 ± 0·92	72
	SDF	0·98 ± 0·25	3·17 ± 0·17	34

Mean value ± standard deviation ($n \geq 3$).

KL fractions of DF contain not only lignin, but also other insoluble components. The contributions of these insoluble constituents to the KL fraction will depend upon the composition of the food being analysed as well as on the physical and chemical interactions between its components.

CONCLUSIONS

The results indicate that:

- The presence of protein during the DF analysis modifies results by increasing the KL fraction value and by altering the content and/or distribution of polysaccharides.
- IDF and SDF fraction distribution depends upon the chemical and physical conditions of the analysis. SDF constituents can be retained in the IDF fraction.
- The results of gravimetric DF quantification do not correspond to actual DF content. Depending upon the sample, the DF value can be over- or undervalued.
- Besides lignin, the KL fraction is made up of other acid-insoluble food constituents and/or their condensed products.

These errors may affect both the enzymatic–gravimetric and the enzymatic–chemical methods. We conclude that the most widely used methods for DF analysis neglect certain factors that can significantly modify their outcomes. Specifically:

- The AOAC official method (Prosky *et al.*, 1988) may be affected by the errors associated with both the gravimetric quantification of DF fractions and the previously reported ethanolic precipitation of SDF (Mañas & Saura-Calixto, 1993).
- The Englyst method (Englyst & Cummings, 1988) may be affected by errors associated with the absence of a protein hydrolysis step as well as by leaving out the lignin quantification and by obtaining the SDF fraction by ethanolic precipitation.

On the basis of these results, it is concluded that, in some cases, the present DF analytical methods are not accurate enough for either commercial or scientific purposes. The quantitative significance of the mentioned errors will depend upon the type of sample. This is a fact that should be taken into account by the analysts.

Since the results of current DF analysis using either the AOAC official method or the Englyst method can be flawed, analysis is suggested of DF with a different methodology: after performing enzymatic treatments, including protease, samples should be centrifuged to obtain the IDF fractions and supernatants dialysed to obtain the SDF fractions; both IDF and SDF fractions should then be acid-hydrolysed so that their constituents can be chemically quantified.

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Table 11. Study of the presence of cellulose, pectins and protein in the Klason lignin fraction

Components (mg dry matter)				Relative proportions	KL (mg) (range of values)	KL–91·36% lignin (% dry matter)
Cellulose	Pectins	Protein	Lignin			
			150	1	137·0 (91·36%)	–
200				1	1·0–1·3	0·57 ± 0·07
	200			1	2·0–2·4	1·10 ± 0·09
		200		1	7·8–8·0	3·95 ± 0·07
200			150	1 : 0·75	138·7–138·9	0·90 ± 0·05
	200		150	1 : 0·75	140·1–140·6	1·67 ± 0·14
		200	150	1 : 0·75	180·6–181·0	21·89 ± 0·09
150	150	200		1 : 1 : 1·3	18·0–31·9	4·99 ± 1·39
150	150		200	1 : 1 : 1·3	198·0–198·9	5·24 ± 0·14
200		150	150	1·3 : 1 : 1	171·0–184·4	11·63 ± 1·92
	200	150	150	1·3 : 1 : 1	236·9–280·0	34·71 ± 6·16

Mean value ± standard deviation ($n \geq 3$).

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