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Ethanolic precipitation: A source of error in dietary fibre determination

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The ethanol precipitation of soluble dietary fibre (SDF) constituents is a common step among modern analytical methods.

This precipitation may involve two possible error sources: non-fibre component co-precipitation and incomplete precipitation of SDF components such as pectins. Both possibilities are studied in the present work.

Citrus pectins and pectin–organic acid (citric and oxalic) mixtures were dissolved in phosphate buffer and precipitated in 78% ethanol at different pH values. Incomplete recovery of pectins (84–89%, depending upon the pH of the solution) and significant co-precipitation of organic acids were observed.

Dialysis has been studied as an alternative method for obtaining SDF. Both dialysis and precipitation techniques were carried out to determine SDF in fruits, legumes and cereals. The SDF values (neutral sugars plus uronic acids) obtained by dialysis were higher in all the samples than the values obtained by precipitation.

These results show the incomplete determination of SDF with the present analytical methods. In addition, gravimetric methods can include a fraction of non-fibre constituents, such as organic acids, as fibre.

INTRODUCTION

Modern methods for dietary fibre (DF) analysis can be generally divided into two groups: enzymatic–gravimetric and enzymatic–chemical techniques.

The most widely used are the enzymatic–gravimetric official AOAC method (Prosky, 1988), approved as the legal or recommended procedure in at least ten countries (Schweizer, 1989), and the enzymatic–chemical Englyst method (Englyst *et al.*, 1982), recommended as the official method in the United Kingdom (Englyst & Cummings, 1988).

There are several methods proposed by different authors which can also be included in these two groups (Schweizer & Würsch, 1979; Selvendran & Du Pont, 1980; Furda, 1981; Theander & Aman, 1982; Asp *et al.*, 1983; Arrigoni *et al.*, 1984; Faulks & Timms, 1985; Brillouet *et al.*, 1988; Li & Andrews, 1988; Jeraci *et al.*, 1989; Lee & Hicks, 1990).

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A common step in DF analysis is ethanol precipitation to determine soluble dietary fibre (SDF) and/or total dietary fibre (TDF). TDF can be determined as insoluble dietary fibre (IDF) plus SDF (Schweizer & Würsch, 1979; Furda, 1981; Asp *et al.*, 1983; Arrigoni *et al.*, 1984; Brillouet *et al.*, 1988) or by difference between TDF and IDF (Aman & Graham, 1987; Englyst & Cummings, 1988; Prosky *et al.*, 1992). In any case, the recovery of SDF by ethanol precipitation is sometimes impractical, either because of the volumes involved or the presence of starch hydrolysis products.

This precipitation may involve two possible error sources: non-fibre component co-precipitation and incomplete precipitation of SDF components.

The co-precipitation of non-fibre constituents may include not only reagents such as buffer, previously added in the analytical method, as has been mentioned by other authors (Lee & Hicks, 1990), but also the co-precipitation of sample components.

With regard to SDF ethanol precipitation, many factors such as the solution pH and ionic strength and

the polysaccharide structure may affect the process to such an extent that it is not quantitative.

The aim of the present study was to check both possibilities. On this basis, standard precipitation experiments were conducted. In parallel, SDF determination by both ethanolic precipitation and dialysis techniques in different foods was performed.

MATERIALS AND METHODS

Materials

Citrus pectins (Sigma P-9135), citric acid (Sigma C-7129) and oxalic acid (Panreac) were used as standards.

Fruit samples (orange pulp and peel, lemon pulp and apple pulp) were wastes obtained after industrial juice extraction. Beans and oat flake samples were purchased from a local supermarket. Citrus samples were washed with 96% ethanol to eliminate pigments, free sugars and organic acids. Oat flakes were previously defatted to a content of less than 5% of fat. All samples were finely ground (<0·5 mm) prior to use.

Reference sugars and galacturonic acid were obtained from Merck (Darmstadt, Germany).

Enzymes (heat-stable α-amylase A-5426, protease P-3910 and amyloglucosidase A-9913) came from Sigma St. Louis, MO, USA

All reagents used were of analytical-grade quality.

Apparatus

Filtration equipment

A Fibertec System E 1023 Filtration Module (Tecator, Höganäs, Sweden) allowing individual collection of filtrates was used. Crucibles with porosity 2 (pore size 40–90 μm) were used with Celite (Fluka) as the filter aid.

GLC apparatus

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

Dialysis equipment

A continuous water-renovation system was used. It consisted of running water that was pre-heated by passing along a 20-m length latex tube immersed in a 40°C water-bath. The pre-heated 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.

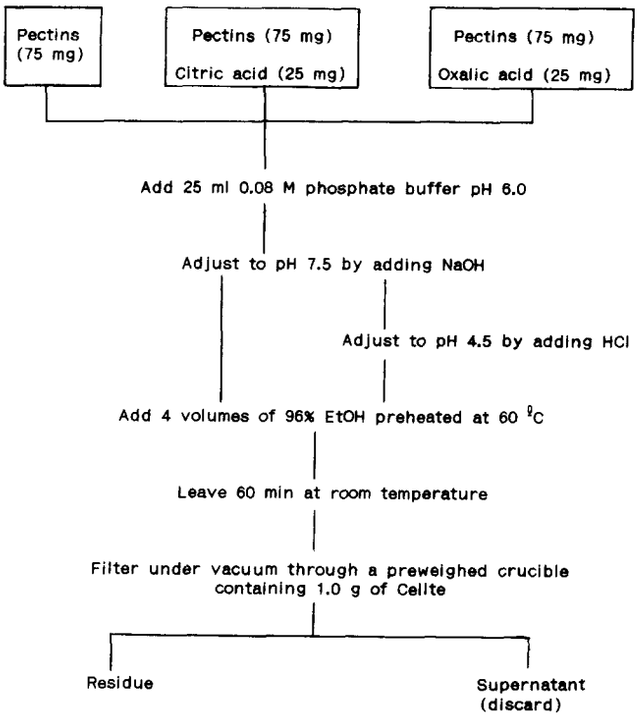

Fig. 1. Scheme for standards precipitation procedure.

Methods

Standard precipitations

Pectins and mixtures of pectins and organic acid (citric and oxalic) were solubilized in phosphate buffer, adjusted to pH 7·5 or 4·5 and precipitated by the addition of 96% EtOH to a final concentration of 78%. Blanks at each studied pH were run through the entire procedure along with samples. Residues were gravimetrically quantified after washings. Blank and ash corrections were performed.

A flow diagram of the procedure is given in Fig. 1.

SDF determination

SDF was determined in fruits, beans and oats. Figure 2 shows a brief scheme of the experiment.

Enzymatic steps and filtration were carried out following the AOAC method conditions (Prosky *et al.*, 1988). Heat-stable α-amylase and amyloglucosidase treatments (enzyme addition and heating) were omitted for citrus samples, because they did not contain starch.

The combined supernatant liquid and water washings were precipitated in 78% ethanol or dialyzed against water.

Dialysis was achieved using a 12 000–14 000 MWCO dialysis bag (Dialysis Tubing Visking 9-32/36 mm Medicell International, Ltd) and the previously described dialysis system.

Precipitated and dialyzed samples were hydrolyzed in 1 M sulphuric acid.

SDF was quantified, in both cases, as neutral sugars (NS) plus uronic acids (UA), by GLC (Englyst & Cummings, 1988) and spectrophotometric (Scott, 1979) techniques, respectively.

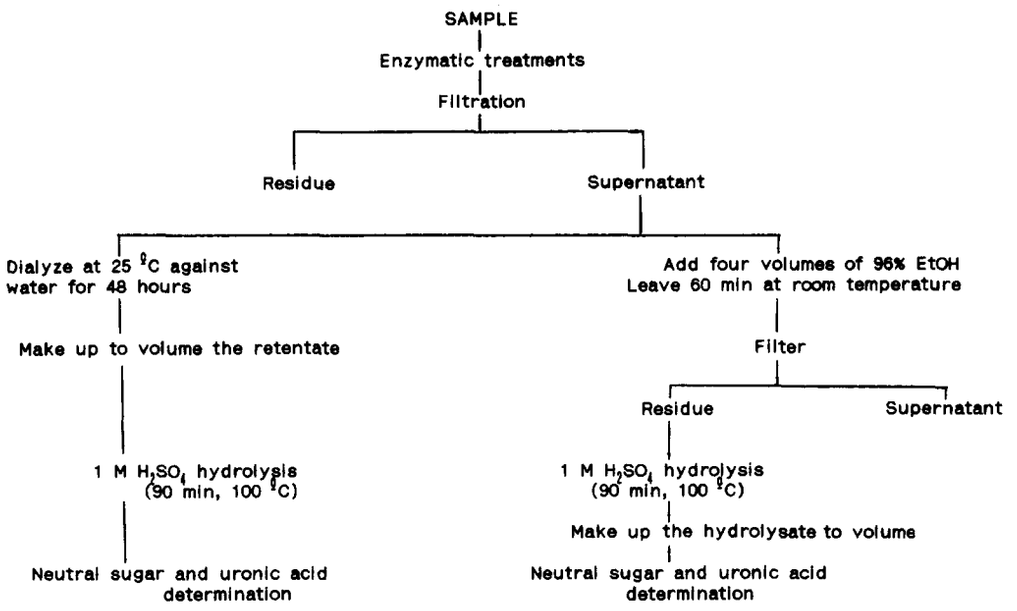

Fig. 2. Scheme for the estimation of soluble dietary fibre.

RESULTS AND DISCUSSION

The 78% ethanol precipitation of citrus pectins, as well as the combination of these with organic acids was studied at a pH of either 7·5 or 4·5. Citric and oxalic acids were chosen because they are commonly present in fruits, and pectins because they are characteristic SDF constituents.

Gravimetric determination of the precipitated fractions were carried out following the AOAC method, omitting the enzyme addition and resistant protein correction because they were not necessary to our study. The ash content of the residues obtained at pH 7·5 were significantly higher (67–70% of total residue) than at pH 4·5 (1–13% of total residue).

The final pH of 4·5 corresponds to starchy foods (Prosky *et al.*, 1988) and pH 7·5 to starch-free samples, which only needed protease treatment (Mañas *et al.*, 1990).

As shown in Table 1, 84% and 89% of the pectins were recovered at pH 4·5 and 7·5, respectively. The lower recovery of pectins corresponded to an acid pH which is the most usual value when SDF precipitation is performed using present analytical methods (pH 4·5: Arrigoni *et al.*, 1984; Prosky *et al.*, 1988; Lee & Hicks, 1990; pH 4·8: Li & Andrews, 1988; pH 5·0: Theander & Westerlund, 1986; pH 5·2: Englyst & Cummings, 1988).

Table 1. Weights of 78% ethanol precipitation residues of pectins and pectins plus organic acids (% dry pectin basis)

pH	Pectins	Pectins + citric acid	Pectins + oxalic acid
7·5	88·9 ± 1·7[a]	91·5 ± 0·4[b]	108·8 ± 4·5[c]
4·5	83·7 ± 0·2[d]	94·0 ± 2·5[e]	104·8 ± 2·8[f]

Mean values of at least three determinations ± standard deviation.
a-d, a-c and *d-f* were significantly different: $P < 0{\cdot}01$.
a-b and *d-e* were significantly different: $P < 0{\cdot}05$.

On the other hand, the pectin–organic acid mixtures always yielded a higher gravimetric residue as compared with the pectins sample: more than 90% with citric acid and more than 100% with oxalic acid. This may be due both to a higher pectin precipitation and/or to a co-precipitation of organic compounds along with SDF constituents. The results obtained with pectin–oxalic acid mixtures clearly indicate that a co-precipitation factor is involved in the process.

It should be noted that these experiments have been designed as a rapid test to check if pectin precipitation was quantitative and if co-precipitation could also affect the results, and the relative influence of each factor is not the aim of this test.

The percentage of pectin losses and/or co-precipitation of other components along with SDF constituents will be different depending upon the sample composition and the analytical conditions (pH, buffer, temperature, etc.).

The errors associated with the gravimetric quantification of SDF by ethanol precipitation may be summarized as:

* Incomplete and variable recovery of soluble polysaccharides according to the solution pH, ionic strength and the presence of different organic acids.
* A residue of organic compounds included in the gravimetric values. When ash correction is performed this organic non-fibre fraction is only partially eliminated.

To avoid these problems, dialysis was studied as an alternative method for obtaining SDF values.

Dialysis conditions were previously optimized, and dialysis and precipitation techniques were both carried out to isolate SDF in fruits, legumes and cereals.

As can be observed in Table 2, SDF (expressed as

Table 2. SDF contents obtained by ethanolic precipitation and by dialysis (% dry matter)

	Orange pulp		Orange peel		Lemon pulp		Apple		Beans		Oats	
	Pp	Dy	Pp	Dy	Pp	Dy	Pp	Dy	Pp	Dy	Pp	Dy
Rha	0·15	0·27	0·15	0·21	0·29	0·47	0·11	0·14	0·03	0·05	0·05	0·08
Fuc	0·05	0·10	0·05	0·08	0·10	0·14	0·05	0·04	tr	0·03	tr	0·03
Ara	1·47	2·43	1·36	2·35	1·78	2·88	1·49	3·22	0·33	0·40	0·24	0·38
Xyl	0·03	0·15	0·05	0·08	0·14	0·16	0·22	0·23	0·11	0·25	0·14	0·20
Man	0·12	0·29	0·11	0·21	0·19	0·34	0·36	0·48	0·13	0·47	0·18	0·29
Gal	1·98	2·23	1·82	2·02	1·25	1·48	1·86	1·92	0·23	0·45	0·15	0·39
Glu	0·17	0·62	0·21	0·39	0·34	0·61	0·37	0·69	0·13	0·50	2·30	3·34
ΣNS[a]	3·97	6·09	3·75	5·34	4·09	6·08	4·46	6·72	0·98	2·15	3·06	4·71
UA[b]	1·58	5·76	1·59	6·99	11·64	28·12	8·27	10·49	0·54	0·68	0·11	0·14
SDF[c]	5·55	11·85	5·34	12·33	15·73	34·20	12·73	17·21	1·52	2·83	3·17	4·85

Mean value of at least three determinations.
Pp = precipitation; Dy = dialysis; tr = trace.
[a] NS = neutral sugars.
[b] UA = uronic acids.
[c] SDF = (NS+UA).

(NS+UA)), is always higher when obtained by dialysis. Precipitation values represent only 43–74% of the dialysis ones, with the biggest differences corresponding to citrus samples (43–47%).

Concerning uronic acid content, it must be pointed out that the highest differences also correspond to citrus, the precipitation values of which were 23–41% of the dialysis ones. Apple pulp, bean and oat flake SDF precipitation values were 79% of the dialysis ones.

The values for total neutral sugar precipitation ranged from 45 to 70% of the dialysis ones.

These results accord with the previous standards experiment, which found incomplete pectin precipitation.

Larm *et al.* (1975) suggested that some polysaccharides might not precipitate with 80% ethanol. Other authors (Amadó & Neukom, 1985) have mentioned that certain arabinogalactans (which along with galacturonans form the major component of the water-soluble pectin fraction) are soluble in 80% ethanol.

In any case, the differences found in the precipitation and dialysis of SDF values seems to be due to the incomplete precipitation of pectins and/or other soluble polysaccharides. Factors such as the solution pH and ionic strength, and the composition and physico-chemical properties of the sample may affect the process.

CONCLUSIONS

The qualitative errors found in experiments carried out with standards (incomplete precipitation of SDF constituents and co-precipitation of non-fibre components) along with quantitative errors in the studied samples (fruits, cereals and legumes), where precipitation values represented only 43–74% as compared with dialysis, indicate that the ethanol precipitation process should not be used in DF analysis to obtain the SDF fraction.

Results obtained by dialysis are comparatively more reliable.

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REFERENCES

Amadó, R. & Neukom, H. (1985). Minor constituents of wheat flour: The pentosans. In *New Approaches to Research on Cereal Carbohydrates*, ed. R. D. Hill & L. Munck. Elsevier Applied Science Publishers B.V., Amsterdam, The Netherlands, pp. 217–30.

Aman, P. & Graham, H. (1987). Analysis of total and insoluble mixed-linked (1-3),(1-4)-β-D-glucans in barley and oats. *J. Agric. Food Chem.*, **35**, 704–9.

Arrigoni, E., Caprez, A., Amadó, R. & Neukom, H. (1984). Gravimetric method for the determination of insoluble and soluble dietary fibres. *Z. Lebensm. Unters. Forsch.*, **178**, 195–8.

Asp, N. G., Johansson, C. G., Hallmer, H. & Siljeström, M. (1983). Rapid enzymatic assay of insoluble and soluble dietary fiber. *J. Agric. Food Chem.*, **31**, 476–82.

Brillouet, J. M., Rouau, X., Hoebler, C., Barry, J. L., Carré, B. & Lorta, E. (1988). A new method for determination of insoluble cell walls and soluble nonstarchy polysaccharides from plant materials. *J. Agric. Food Chem.*, **36**, 969–79.

Englyst, H. N. & Cummings, J. H. (1988). Improved method for measurement of dietary fiber as non-starch polysaccharides in plant foods. *J. Assoc. Off. Anal. Chem.*, **71**, 808–14.

Englyst, H., Wiggins, H. S. & Cummings, J. H. (1982). Determination of the non-starch polysaccharides in plant foods by gas–liquid chromatography of constituent sugars as alditol acetates. *Analyst*, **107**, 307–18.

Faulks, R. M. & Timms, S. B. (1985). A rapid method for determining the carbohydrate component of dietary fibre. *Food Chem.*, **17**, 273–87.

Furda, I. (1981). Simultaneous analysis of soluble and insoluble dietary fiber. In *Basic and Clinical Nutrition*, Vol. 3. *The Analysis of Dietary Fiber in Food*, ed. W. P. T. James & O. Theander. Marcel Dekker Inc., New York, USA, pp. 163–72.

Jeraci, J. L., Lewis, B. A., Van Soest, P J. & Robertson, J. B. (1989). Urea enzymatic dialysis procedure for determina-

tion of total dietary fiber. *J. Assoc. Off. Anal. Chem.*, **72**, 677–81.

Larm, O., Theander, O. & Aman, P. (1975). Structural studies on a water-soluble arabinan isolated from rapeseed (*Brassica rapus*). *Acta Chem. Scand., Ser. B*, **B29**, 1011–4.

Lee, S. C. & Hicks, V. A. (1990). Modifications of the AOAC total dietary fiber method. In *New Developments in Dietary Fiber. Physiological Physicochemical and Analytical Aspects*, ed. I. Furda & C. J. Brine. Plenum Press, New York, USA, pp. 237–44.

Li, B. W. & Andrews, K. W. (1988). Simplified method for determination of total dietary fiber in foods. *J. Assoc. Off. Anal. Chem.*, **71**, 1063–4.

Mañas, E., Abia, R. & Saura-Calixto, F. (1990). Some problems associated with the determination of dietary fibre in citrus by the AOAC method. In *Dietary Fibre: Chemical and Biological Aspects*, ed. D. A. T. Southgate, K. Waldron, I. T. Johnson & G. R. Fenwick. The Royal Society of Chemistry, Cambridge, UK, 124–9.

Prosky, L., Asp, N. G., Schweizer, T. F., DeVries, J. W. & Furda, I. (1988). Determination of insoluble, soluble, and total dietary fiber in foods and food products: Interlaboratory study. *J. Assoc. Off. Anal. Chem.*, **71**, 1017–23.

Prosky, L., Asp, N. G., Schweizer, T. F., DeVries, J. W. & Furda, I. (1992). Determination of insoluble and soluble dietary fiber in foods and food products: Collaborative study. *J. Assoc. Off. Anal. Chem.*, **75**, 360–7.

Schweizer, T. F. (1989). Dietary fibre analysis. *Lebensm. Wiss. u.-Technol.*, **22**, 54–9.

Schweizer, T. F. & Würsch, P. (1979). Analysis of dietary fibre. *J. Sci. Food Agric.*, **30**, 613–9.

Scott, R. W. (1979). Colorimetric determination of hexuronic acids in plant materials. *Anal. Chem.*, **51**, 936–41.

Selvendran, R. R. & Du Pont, M. S. (1980). Simplified methods for the preparation and analysis of dietary fibre. *J. Sci. Food. Agric.*, **31**, 1173–82.

Theander, O. & Aman, P. (1982). Studies on dietary fibre. A method for the analysis and chemical characterisation of total dietary fibre. *J. Sci. Food Agric.*, **33**, 340–4.

Theander, O. & Westerlund, E. A. (1986). Studies on dietary fiber. 3. Improved procedures for analysis of dietary fiber. *J. Agric. Food Chem.*, **34**, 330–6.