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Determination of Dietary Fibre in Cider Wastes. Comparison of Methods

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ABSTRACT

Cider wastes are rich in polysaccharides and can be considered a suitable source of dietary fibre (DF). The measurement of DF as 'non-starch polysaccharides' (48·3% dry matter), and as 'non-starch polysaccharides plus lignin', (63·3%), is performed by spectrophotometric and AOAC procedures. A good agreement between both methods for polysaccharide content is found. Correction for condensed tannins (3·1% dry matter) is made. The Klason lignin residue contains protein and condensed tannins.

INTRODUCTION

The availability of high quality foods with high dietary fibre (DF) contents is of key importance for obtaining changes in fibre intakes according to general dietary recommendations to adults in Western societies and diets recommended for the treatments and prevention of several diseases such as hypertension, atherosclerosis and diabetes (Vahouny & Kritchevsky, 1986). Subsequently, new high fibre food products and ingredients have been developed by some companies. Cereals are the basic material for these products. Nevertheless, cereals contribute 35–60% to the present total DF intake in North European countries, while the corresponding percentage from fruits is only 7–15% (Bingham, 1985, 1987). Thus, it would be interesting to find new sources from fruits for high DF products.

Over 2 500 000 tons of apples are dedicated to cider production in Europe (Saura-Calixto, 1975). After grinding and extracting the juice for

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fermentation, the remaining apple pulp is rich in DF. These wastes are analysed in the present paper.

Methods for DF analysis can be divided into two groups: (1) gravimetric methods in which the DF is determined by weighing after removal of non-fibre components; the results are expressed as non-starch polysaccharides plus lignin (Theander & Westerlund, 1986; Asp, 1987). (2) methods by which DF content is expressed as non-starch polysaccharides. After removal of starch and acid hydrolysis, the neutral sugar and uronic acids content, determined spectrophotometrically or by GLC, are summed to obtain the total DF (Englyst & Cumming, 1984; Faulks & Timms, 1985; Englyst & Hudson, 1987). Both methods are used in this work for comparison.

On the other hand, the influence of condensed tannins in DF analysis was previously reported (Saura-Calixto, 1987, 1988). The presence of these compounds in cider wastes is also considered.

MATERIALS AND METHODS

Samples

Cider wastes were supplied by the factory Sidra El Gaitero, S. A. (Villaviciosa, Asturias, Spain). The material was apple pulp containing peels and some seeds. Samples were freeze-dried, and then homogenized and ground to particles of $\leq 0{\cdot}5$ mm.

Reagents and equipment

All chemicals were analytical grade. The enzyme preparations were: FiberZym Kit with heat-stable alpha-amylase, protease and amyloglucosidase (Novo Biolab, Bagsvaerd, Denmark), protease P-5380 (Sigma Chemicals, St. Louis, USA) and pullulanase (Boehringer, Mannheim, West Germany).

The following equipment was employed: Fibertec System E, Kjeltec 1030 Autoanalyzer, Soxtec System 1040 Extraction unit (Tecator, Hoganas, Sweden); Telstar freeze-dryer, (Telstar, S. A., Barcelona, Spain); Perkin-Elmer Lambda 5 spectrophotometer, (Perkin-Elmer Corp., Norwalk, USA).

DF determination

Analytical schemes of the different methods used are shown in Figs 1 and 2. The procedures are briefly described below.

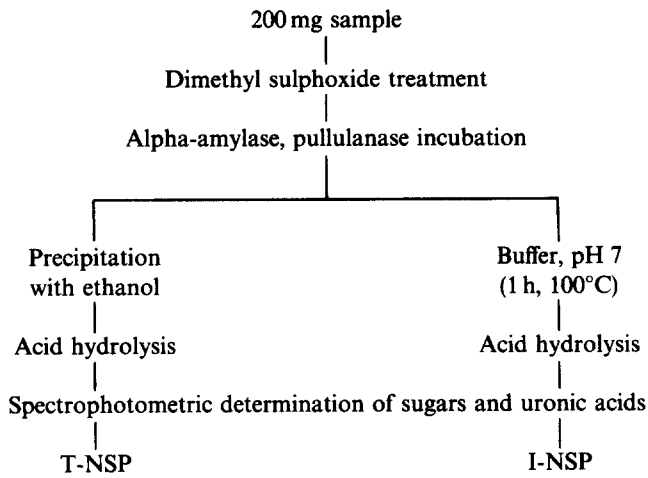

Fig. 1. Analytical scheme for the spectrophotometric method for dietary fibre as non-starch polysaccharides (Procedure A).

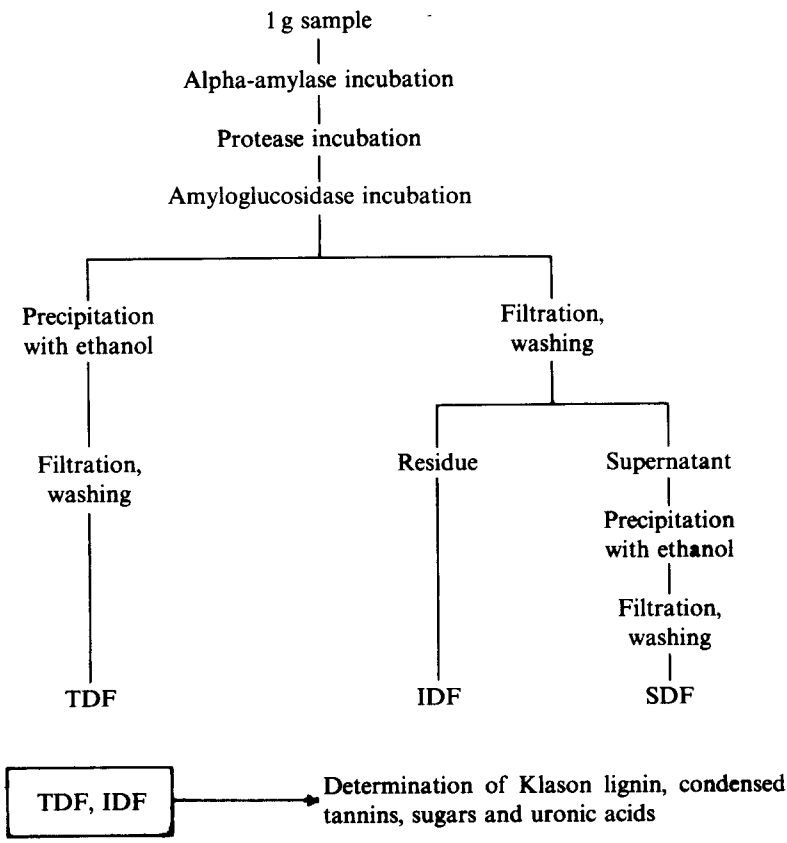

Fig. 2. Analytical scheme for enzymatic gravimetric methods for dietary fibre (Procedures B and C).

Procedure A

Two hundred milligram samples were treated with 2 ml of dimethyl sulphoxide (1 h, 100°C) and then incubated with alpha-amylase-pullulanase solution (pH: 5·2, 42°C, 16 h). The residue obtained by precipitation with ethanol was, after washing, successively treated with 12M H_2SO_4 (1 h, 35°C) and 1M H_2SO_4 (2 h, 100°C). Aliquots were taken from the hydrolysate to determine sugars and uronic acids content by spectrophotometric analysis. This content corresponds to total non-starch polysaccharides (T-NSP).

Treatments with buffer pH:7 (1 h, 100°C) were performed, instead of ethanol precipitation, in some samples, to determine the insoluble non-starch polysaccharides (I-NSP). Soluble non-starch polysaccharides (S-NSP) were calculated by difference.

More experimental details are described in the literature (Cummings *et al.*, 1985; Englyst & Hudson, 1987).

Procedure B

The experimental conditions described by Prosky *et al.*, (1985), were followed. One gram of sample was treated with heat-stable alpha-amylase (100°C, pH:6, 30 min), protease (60°C, pH:7·5, 30 min) and amylo-glucosidase (60°C, pH:4·5, 30 min). The soluble fibre components were precipitated by adding 95% ethanol. After filtration and washing with 78% ethanol, 95% ethanol and acetone, the dry residue corresponds to TDF. Determination of ash and protein were carried out in the residue and in the blank for the corresponding corrections.

Procedure C

After similar enzymatic treatment to the procedure B, filtration and washing with distilled water, 95% ethanol and acetone were carried out. The residue corresponds to IDF. Filtrate and washings were saved and precipitated by adding 4 volumes of 95% ethanol. The corresponding residue (SDF) was washed with 78% ethanol, 95% ethanol and acetone. Total dietary fibre (TDF) was determined by summing SDF and IDF. Corrections for protein and ash on both residues were also performed.

After addition of the first buffer to the samples in procedures A, B and C, the pH dropped about 2 units below the buffer pH because of the high organic acids content of the cider wastes. Checking and adjusting the pH before adding the first enzyme is necessary.

Determination of DF constituents

The analytical methods used to determine the fiber constituents on dry cider wastes and on DF results are referenced below.

Analyses of sugars were carried out by using anthrone/thiourea as reagent and glucose as standard following the conditions described by Southgate (1976). Uronic acid determinations were performed by the Blumenkrantz & Asboe-Hansen method (1973) (*m*-hydroxy-diphenyl as reagent and galacturonic acid as standard).

Condensed tannins were calculated reading absorbances at 550 nm of anthocyanidin solutions obtained after 5% HCl–BuOH treatment (3 h, 100°C) of samples. Pure vegetable condensed tannins, supplied by Nestlé SA, were used as standard (Reed *et al.*, 1982).

Klason lignin was gravimetrically determined after acid treatments of the samples (12M H_2SO_4; room temperature; 3 h; dilution to 0·358M H_2SO_4 and reflux for 6h), (Theander & Aman, 1980).

Spectrophotometric determinations of protein on 0·5N NaOH solution of the original materials were made by the Lowry method (Lowry *et al.*, 1951).

Kjeldahl protein (factor: 6·25), ash and oil were determined following standard procedures.

RESULTS AND DISCUSSION

Determination methods

The moisture content of the original material was 81·4%. Data of this section will be referred to dry matter.

The results obtained by procedure A are shown in Table 1. Sugars and

TABLE 1
Content of Dietary Fibre Determined Spectrophometrically as Non-Starch Polysaccharides (% dry matter).[a] Procedure A

	T–NSP	*I–NSP*	*S–NSP*[b]
Neutral sugars	32·80 ± 1·30	28·40 ± 0·38	4·40
Uronic acids	15·50 ± 0·79	5·56 ± 0·14	9·94
	48·30	33·96	14·34

[a] Mean values of three determinations ± standard deviation.
[b] Determined by difference.

uronic acids are jointly quantified by using dinitrosalicylic acid as reagent (Hosteller *et al.*, 1951; Botle & Gillart, 1958), in the colorimetric method described by Englyst & Hudson (1987). We determined separately those components with different and specific properties (Nyman *et al.*, 1986; Eastwood *et al.*, 1986). The analysis of sugars with anthrone-thiourea (Southgate, 1976) and uronic acids with *m*-hydroxydiphenyl (Blumenkrantz & Asboe Hanson, 1973), are also rapid procedures that can be adopted for routine measurements.

Table 2 shows the DF contents obtained by AOAC procedures (Prosky *et al.*, 1985). The good agreement between the values of TDF obtained by procedures B and C can be pointed out. This methodology permits the isolation of total, insoluble and soluble DF residues that can be used for further studies on properties or composition.

Treatments of IDF and TDF with HCl/BuOH show the presence of appreciable amounts of condensed tannins in our samples. These compounds form complexes with protein, and probably they could be considered as fibre constituents (expressing DF as NSP, lignin and tannins) because they escape digestion. The experimental DF values are corrected for tannins in Table 2.

The contents of sugars and uronic acids of TDF and IDF residues, determined after hydrolysis, are in accord with the results of procedure A (Tables 1 and 2).

The difference between the experimental DF values of Tables 1 and 2

TABLE 2
Contents of Dietary Fibre Determined by AOAC Methods and Sugars and Uronic Acids of TDF and IDF Residues (% dry matter)[a]

	Procedure A	*Procedure B*		
	TDF	*IDF*	*SDF*	*TDF*[b]
Experimental value (polysaccharides, lignin and condensed tannins)	66·38 ± 0·37	51·33 ± 0·38	14·18 ± 0·31	65·51
Condensed tannins	3·04 ± 0·10	3·10 ± 0·10	—	3·10
Polysaccharides plus lignin	63·34	48·29	14·18	62·47
Neutral sugars	32·60 ± 1·10	27·50 ± 0·32		
Uronic acids	15·45 ± 0·65	5·75 ± 0·11		
	48·10	33·25		

[a] Mean values of five determinations ± standard deviation.
[b] Calculated as IDF plus SDF.

TABLE 3
Klason Lignin Content of Cider Wastes (Experimental and by Difference Values)

Calculated as:	*% Dry matter*
TDF (B)—sugars and uronic acids	15·24
IDF (C)—sugars and uronic acids	16·04
TDF (B)—T-NSP (A)	15·04
IDB (C)—I-NSP (A)	14·33
Mean value	15·16
Experimental value:	20·01 ± 1·00
Protein content of Klason lignin residue	1·93 ± 0·07
Klason lignin plus phlobaphenes	18·17

corresponds to lignin because of the different fibre definition (NSP and NSP plus lignin). Lignin content can be estimated by subtracting the polysaccharide content (sugars and uronic acids) from the weight of TDF and IDF residues, as indicated in Table 3. Nevertheless, the experimental value of Klason lignin was higher than that estimated by difference. Condensed tannins are converted into inert phlobaphenes, but not dissolved, during the acid treatment to obtain Klason lignin residue (Tamir *et al.*, 1971), which must include phlobaphenes and probably, associated protein. Kjeldahl determinations on these residues showed 9·6% protein (1·93% referred to original dry matter). Subsequently, the experimental values should be corrected for protein and tannins.

The protein determined spectrophotometrically on 0·5N NaOH extracts of cider wastes was 2·97%. This content coincides approximately with the protein fraction digested during protease incubation. The protein determined on dry samples by Kjeldahl procedure was 5·4%. This value includes both digestible and indigestible protein.

Dietary fibre source

Cider wastes can be considered a good raw material for high DF products. Probably, these subproducts could be directly used, after drying, as fibre source because the dry material contains low amounts of soluble sugars (2·9%), oil (2·7%), protein (5·4%) and ash (2·1%).

Good properties as intestinal regulators, measureable fermentability and lowering effects on cholesterol could be expected from these samples because of their high contents of insoluble fibre and appreciable amounts of soluble fibre and uronic acids.

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