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Dietary Fibre Complex in a Sample Rich in Condensed Tannins and Uronic Acids

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ABSTRACT

The content and composition of dietary fibre in Spanish sainfoin (Hedysarum coronarium), *a leguminous plant containing appreciable amounts of condensed tannins and uronic acids, has been determined by different methods.*

Dietary fibre, obtained by enzymatic procedures and detergent treatments, contains tannins and protein because of protease inhibition and the protein–tannin complexes prevents the complete removal of these constituents in vegetable matter. Different amounts of uronic acids are lost depending on the method used; the highest percentage of pectins remaining in fibre residues—about 90·0% of total content—is observed when an enzymatic method is used.

The results obtained by an enzymatic method, neutral detergent treatment, crude fibre determination and dietary fibre calculated by difference are discussed.

INTRODUCTION

The recognition of the relationship between the content and chemical composition of dietary fibre in foods and its physiological properties requires accurate analytical methodology. The different methods proposed (James & Theander, 1981; Birch & Parker, 1983; Cummings *et al.*, 1985) are principally based on enzymatic procedures (Asp *et al.*, 1983; Arrigoni *et al.*, 1984; Faulks & Timms, 1985), detergent solution treatments (Van Soest, 1967; Robertson & Van Soest, 1977) or determinations by difference (Katan

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& Van de Bovenkamp, 1981). A discussion of problems associated with the analysis of dietary fibre is provided by Selvendran & Du Pont (1984).

Usually, samples are first defatted and then treated specifically to remove water-soluble substances, starch and intracellular protein. The neutral detergent fibre (NDF) system is useful for samples where cellulose and hemicellulose form the major constituents, but a loss of pectic substances and incomplete removal of starch occurs. Soluble and insoluble parts of dietary fibre—SDF and IDF—are obtained by enzymatic methods, although experimental conditions and the presence of some plant components may affect the composition of both fractions. In difference methods there is no distinction between soluble and insoluble dietary fibre and these only provide an estimate of the total fibre content.

The possible presence of tannins is not considered in most of the available DF methods but, when these vegetable constituents are not minor components in samples, important qualitative and quantitative errors could be produced. On the other hand, the study of polyuronic substance recovery in the experimental treatments of the different methods is important, especially when the content is appreciable or when linked with other fibre components.

Spanish sainfoin (*Hedysarum coronarium*) is a leguminous plant cultivated in Menorca-Baleares, Spain, of which the forage (stems plus leaves) contains appreciable amounts of condensed tannins and uronic acid-containing polymers.

In the present paper, different methods were employed for dietary fibre determination in order to observe the influence of the above-mentioned components upon the analytical results.

EXPERIMENTAL

Sampling

Fresh forage of Spanish sainfoin, planted in the experimental field of Escuela de Capacitación Agraria de Mahón (Menorca, Spain) were collected in March, 1985. Plants were 80 cm in height and the weight ratio between stems and leaves, determined on the basis of dry matter, was 79:21. Moisture (85·0%) was removed by drying at 65°C under vacuum for 2 days and samples were then homogenized and ground to particles of $\leq 0{\cdot}3$ mm.

Analytical methods

One gram of sample was successively treated with Termamyl-60 L, protease P-5380 and amyloglucosidase and SDF and IDF were determined

gravimetrically after centrifugation and dialysis. Experimental conditions described by Arrigoni *et al.* (1984) were followed.

NDF values were obtained by refluxing the samples (3 g) with sodium laurylsulfate (pH 7·0) solution for 1 h, following the procedures of Van Soest (1967). Treatments with 0·255N H_2SO_4 and 0·313N NaOH were performed to determine crude fibre (CF) content (AOAC, 1980).

Protein contents were calculated as the sum of amino acids after protein hydrolysis (6N HCl, 120°C, 24 h, under N_2) and amino acid analyses were performed with a Liquimat III Analyzer under specific conditions. In some experiments, in order to make quicker assays, the modified ninhydrin photometric method of Moore & Stein (1954) was also used to determine total amino acid content by using leucine as standard.

Water-soluble pectins were determined in aqueous extracts (60°C, 30 min) and the uronic acid-containing polymers remaining in the water-insoluble residue and fibre fractions were solubilized by Saeman hydrolysis (Selvendran *et al.*, 1979); 20 mg was wetted with 1 ml of 72% H_2SO_4 and left

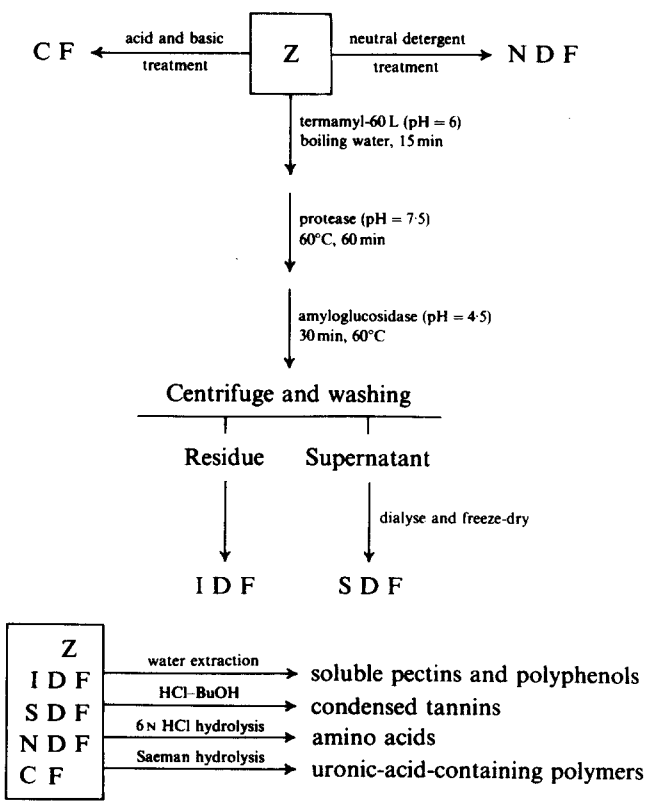

Fig. 1. Scheme of experimental methodology (Z = samples of Spanish sainfoin forage, dried and homogenized).

at 20°C for 3 h and then diluted with distilled water to 10 ml. Quantitative determinations of pectins were performed on both aqueous solutions and solutions obtained after Saeman hydrolysis. In order to avoid neutral sugar interference, the spectrophotometric methods proposed by Blumenkrantz & Asboe-Hansen (1973)—*m*-hydroxydiphenyl as reagent and galacturonic acid as standard—were used.

Water-soluble polyphenols were spectrophotometrically determined with Folin-Denis reagent and D-cathequin as standard (Swain & Hillis, 1959). Condensed tannin contents were calculated reading absorbances at 550 nm of anthocyanidin solutions obtained after 5% HCl–BuOH treatment (10 ml, 3 h, 100°C) of samples (20 mg). Different amounts of pure vegetable condensed tannins, supplied by Nestlé SA, were treated under the same conditions to obtain standard curves (Haslam, 1966; Reed *et al.*, 1982).

A scheme of the experimental methodology is shown in Fig. 1.

RESULTS AND DISCUSSION

Table 1 shows the values for dietary fibre obtained and its major constituents. The fibre value determined by enzymatic methods (SDF + IDF) is appreciably higher than the detergent fibre value (NDF). As can be observed, a significant part of cell-wall constituents (unavailable carbohydrates-lignin) are solubilized during detergent treatment.

CF content is markedly lower than the rest of the fibre values. In a previous paper (Saura-Calixto *et al.*, 1983) the amounts of hemicellulose, cellulose and lignin lost during CF treatments were compared with NDF values for different samples (between 20% and 70%). In this case the content of unavailable carbohydrates-lignin in CF represents 59·1% of the content in NDF and 42·1% of the enzymatic value.

Determination of DF by difference is considered below.

Pectic substances have been reported to be particularly effective in lowering serum cholesterol. High amounts of these compounds are also lost during NDF and CF treatments.

Percentages of protein, pectins and tannins—referred back to vegetable dry matter—found in fibre residues are shown in Table 2. The presence of important amounts of protein in SDF and IDF fractions is related to the presence of condensed tannins in the samples. Polyphenolic compounds form molecular complexes with protein (Kumar & Singh, 1984), making extraction difficult and, in addition, can produce inhibition of proteases (Humphries, 1980; Griffiths & Moseley, 1980). Both these factors contribute to protein retention in enzymatic fibre (41·4%). In NDF only the first factor

TABLE 1
Content and Composition of Dietary Fibre Obtained by Different Methods in Spanish Sainfoin (% dry matter)[a]

	1. *Experimental value of dietary fibre*	2. *Protein*	3. *Pectins*		4. *Polyphenols*		5. *Unavailable carbohydrates-lignin (b)*	6. *Unavailable carbohydrates-lignin-pectins*[b]
			Soluble	*Insoluble*	*Soluble*	*Condensed*		
Enzymatic method								
Soluble dietary fibre (SDF)	13·8 ± 0·5	1·3 ± 0·1	5·2 ± 0·2	—	0·3 ± 0·0	—	7·0	12·2
Insoluble dietary fibre (IDF)	54·6 ± 0·4	2·1 ± 0·1	—	7·8 ± 0·3	—	5·6 ± 0·5	39·1	46·9
Total	68·4 ± 0·9	3·4 ± 0·2	5·2 ± 0·2	7·8 ± 0·3	0·3 ± 0·0	5·6 ± 0·5	46·1	59·1
Neutral detergent fibre (NDF)	38·9 ± 0·4	1·9 ± 0·1	—	2·9 ± 0·2	—	1·3 ± 0·1	32·8	35·7
Crude fibre (CF)	20·8 ± 0·3	—	—	1·4 ± 0·1	—	—	19·4	20·8
Dietary fibre by difference							46·5	61·2

[a] Mean value of five determinations ± standard deviation.
[b] Determined by difference (5 = 1 − 2 − 3 − 4; 6 = 1 − 2 − 4).

TABLE 2

Protein, Pectin and Tannin Contents in Spanish Sainfoin (% dry matter) and Percentage of these Constituents remaining in Fibre Residues

	Protein	*Pectic substances*			*Polyphenols*		
		Soluble	*Insoluble*	*Total*	*Soluble*	*Condensed*	*Total*
Spanish Saintoin Forage	8·2 ± 0·4	1·7 ± 0·1	13·0 ± 0·9	14·7 ± 1	1·1 ± 0·1	7·0 ± 0·6	8·1 ± 0·7
Soluble dietary fibre (SDF)	15·8			35·7			3·7
Insoluble dietary fibre (IDF)	25·6			53·1			69·1
Total	41·4			88·8			72·8
Neutral detergent fibre (NDF)	23·2			19·7			16·0
Crude fibre (CF)	—			9·5			—

is operating and the protein retention is lower (23·2%). Removal of protein in enzymatic procedures occurs during dialysis, which indicates some protease activity.

A considerable proportion (72·8%) of the polyphenols remains in the enzymatic fibre residues. The remainder is degraded by the acid treatments and high temperatures used during the corresponding enzymatic assays and is lost during dialysis. Neutral detergent treatment is a more efficient procedure to remove condensed tannins (see Table 2). Reed *et al.* (1982) found that NDF obtained from cassava leaf blades also contains tannins and protein. CF procedures give a total solution of polyphenols and protein.

As uronic acid-containing polymers are considered desirable constituents of fibre, an enzymatic method is the most suitable procedure to avoid losses of these components. In our samples nearly 90% of total pectins remains in both SDF and IDF. A portion of the pectins linked with other fibre constituents is solubilized during enzymatic assays and appears in SDF. A further portion of the soluble pectins is lost during dialysis. Losses of pectins in NDF and CF methods are very high, as can be observed in Table 2.

Katan & van de Bovenkamp (1981) calculated the amount of dietary fibre by subtracting the contents of ash, protein, starch and lipids from the weight of the methanol/ether-insoluble fraction in freeze-dried food products. By using an analogous procedure in our samples (dried homogenized forage), tannin content must also be subtracted, in addition to the components cited. Thus:

$$\begin{aligned} DF = 100 - [&\text{protein}(8{\cdot}2) + \text{water-soluble sugars}(8{\cdot}0) \\ &+ \text{ash}(10{\cdot}9) + \text{oil}(3{\cdot}6) + \text{tannins}(8{\cdot}1) + \text{starch}(-)] \\ DF = 100 - 38{\cdot}8 &= 61{\cdot}2 \end{aligned}$$

The contents of water-soluble sugars, ash, oil and starch cited in this expression were determined on dry Spanish sainfoin by standard procedures (AOAC, 1980). Protein and tannin determinations are reported above.

This DF value is comparable with total dietary fibre content (SDF + IDF) after subtraction of protein and tannins in both fractions (59·1%). DF calculated by difference could even be considered closer to the actual value because losses of soluble pectins in enzymatic methods are not included.

Other possible minor cell-wall constituents such as cuticular waxes and minerals are not considered in this work. Schweizer *et al.* (1984) reported that much of the ash content of dietary fibre is removed during dialysis.

In conclusion, the content and physiological properties of dietary fibre obtained by enzymatic or detergent methods may be underestimated

because of the presence of condensed tannins and protein remaining after protease or detergent treatments. When the content of tannins is appreciable, previous removal, or, at least, later subtraction, of these substances, should be performed. The enzymatic method is the most suitable procedure to avoid losses of pectins.

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