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Klason Lignin, Condensed Tannins and Resistant Protein as Dietary Fibre Constituents: Determination in Grape Pomaces

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ABSTRACT

Dietary fibre (DF) determinations were carried out on grape pomaces by AOAC and spectrophotometric procedures. Insoluble dietary fibre (IDF) and Klason lignin (KL) residues contained appreciable amounts of condensed tannins (CT) and resistant protein (RP). The presence of CT and RP in the residues obtained after the successive action of amylase, protease and amyloglucosidase and chemical treatments with H_2SO_4 and HCl-triethyleneglycol, together with similar data previously reported for other samples could be considered in a wider definition of the DF complex as 'indigestible polysaccharides, phenolic polymers and resistant protein'. The term 'phenolic polymers' includes both lignin and CT.

INTRODUCTION

The generally accepted definition of dietary fibre (DF) includes polysaccharides and lignin that are not digested or absorbed in the human small intestine (Asp, 1987). This definition includes the main endogenous components of plant materials in the diet which are resistant to digestion. On this basis, methods to determine fibre contents have been proposed (Prosky *et al.*, 1985, 1988). Other components such as lactulose, resistant starch, condensed tannins and indigestible protein are not hydrolysed by digestive enzymes, remaining in fibre residues (Bingham, 1987).

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Some authors define DF exclusively as non-starch polysaccharides (NSP), reporting the corresponding analytical methods (Englyst & Hudson, 1987; Englyst & Cummings, 1988). Controversial points of view on the DF concept have been published recently (Asp *et al.*, 1988; Trowell, 1988).

In addition to the determination of DF content, the knowledge of both the composition of DF and the links between the major constituents are important because of their influence on physiological properties.

The condensed tannins (CT)—proanthocyanidin polymers—are widely distributed in higher plants and occur in significant concentrations in vegetables utilized as human foods. They are polymers formed by flavan-2-ol and flavan-3,4-diol units and form effective cross-links with protein and other molecules (Haslam, 1966; Bate-Smith, 1973; Kumar & Singh, 1984). Inhibition of digestive enzymes by these compounds has been reported (Tamir & Alumot, 1969; Griffiths & Moseley, 1980).

The effect of CT in the analysis of DF was considered in previous papers (Saura-Calixto, 1987, 1988). Significant amounts of CT and resistant protein (RP) were found in DF residues. Subtraction of CT from DF values was recommended to obtain accurate results corresponding to the DF definition as 'indigestible polysaccharides plus lignin'. Alternatively, the consideration of CT as fibre constituents was suggested.

However, CT, lignin and indigestible protein are closely related because indigestible complexes of these substances are common in vegetables. The objectives of this study are to determine the presence of CT and RP in DF residues of grape pomaces after enzymatic and chemical treatment and to consider the possible inclusion of these compounds as fibre constituents.

MATERIALS AND METHODS

A brief scheme of the determinations performed is shown in Fig. 1. Experimental details are described below.

Materials

The samples corresponded to the grape varieties Airen and Garnacha, crushed to obtain white and red wine, respectively. The materials (white grape pomace, WGP, and red grape pomace, RGP) were supplied by Spanish wineries (Cooperativa de Manzanares, Ciudad Real and Cooperativa del Najerilla, La Rioja).

Fresh samples were freeze-dried, homogenized and ground to $\leq 0{\cdot}5$ mm particles.

FiberZym kits with heat-stable amylase, protease and amyloglucosidase

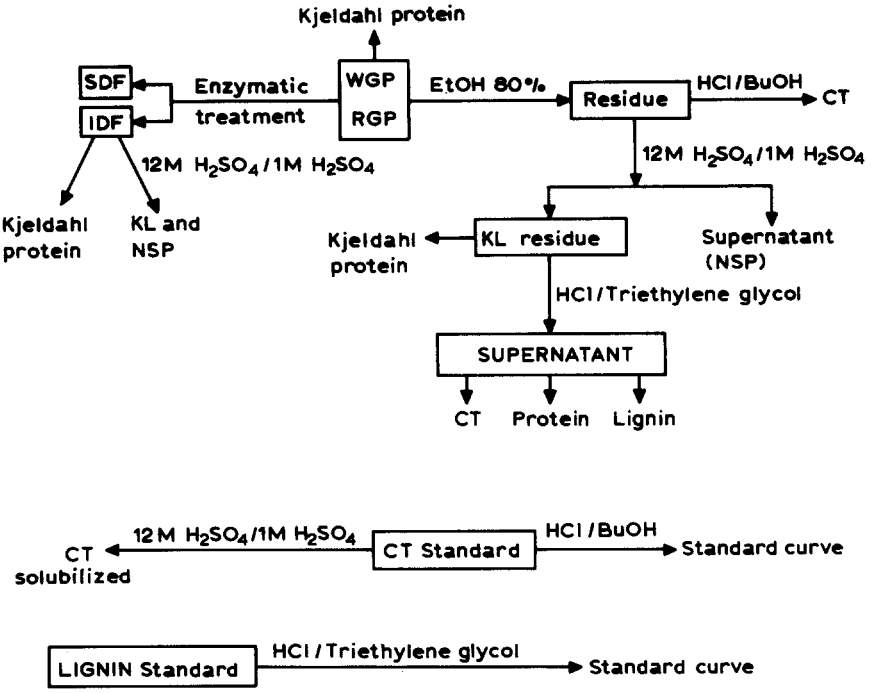

Fig. 1. Brief scheme of the determinations performed.

(NovoBiolab, Bagsvaerd, Denmark) were used for DF determinations. All chemicals were analytical grade. The equipment employed was: Tecator Fibertec System E (DF analysis); Perkin Elmer Lambda 2 UV/VIS spectrophotometer; Tecator 1030 Kjeltec Autoanalyzer (nitrogen determination).

Dietary fibre (AOAC method)

The method was based on the enzymatic removal of starch and protein from the material and separation into soluble and insoluble fractions by filtration and precipitation. The experimental procedure described in the AOAC method was followed (Prosky *et al.*, 1988).

One gram of sample was successively treated with heat-stable α-amylase (100°C, pH = 6, 30 min) protease (60°C, pH = 7·5, 1 h) and amyloglucosidase (60°C, pH = 4·5, 30 min). Then, filtration and washing with distilled water, 95% ethanol and acetone were carried out. The residue corresponds to insoluble dietary fibre (IDF). Four volumes of 95% ethanol were added to the filtrate and washings. The precipitate, soluble dietary fibre (SDF), was washed with 78% ethanol, 95% ethanol and acetone.

Total dietary fibre (TDF) was determined by summing SDF and IDF.

Klason lignin and non-starch polysaccharides

Klason lignin (KL) was determined gravimetrically after acid treatment of the IDF residue by previously established conditions (12M H_2SO_4, 20°C, 3 h; dilution to 1M H_2SO_4 and refluxing for 2 h). Aliquots were taken from the hydrolyzate and sugars and uronic acids were determined spectrophotometrically. Analyses of sugars were carried out with anthrone/thiourea as reagent and glucose as standard (Southgate, 1976). The absorbances of the test solution–anthrone mixtures were measured at 620 nm. Uronic acid determinations were performed by the Blumenkrantz and Asboe-Hansen method (1973) (*m*-hydroxy-diphenyl as reagent and galacturonic acid as standard; absorbance measurements: 520 nm). The sum of sugars and uronic acids corresponds to the insoluble NSP content.

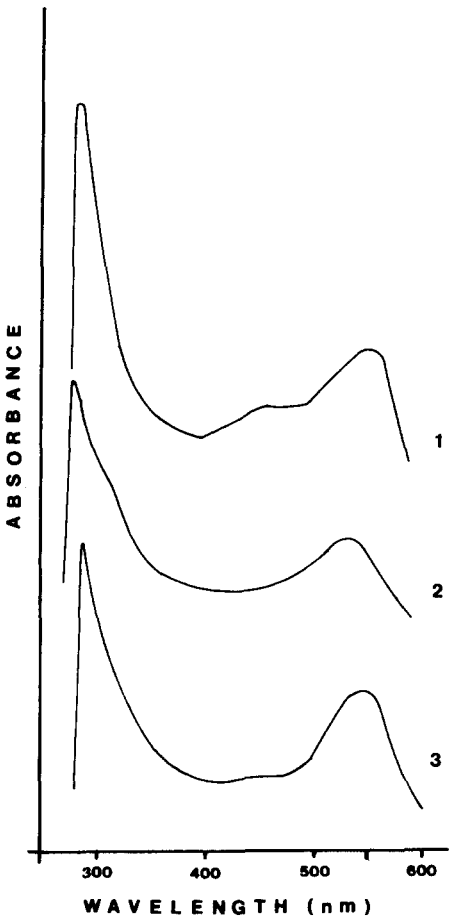

Fig. 2. UV/VIS absorption spectra of anthocyanidin solutions obtained from CT standard (1), white grape pomace (2) and red grape pomace (3).

Portions of dry grape pomaces were treated with 80% ethanol (1 h, 60°C) and the residues were treated similarly to the IDF residues to determine sugars and uronic acids in the acid hydrolyzates. The results correspond to the total NSP because grape pomaces do not contain starch.

Condensed tannins, lignin and protein

Condensed tannins were calculated from the absorbance at 550 nm of anthocyanidin solutions obtained after 5% HCl-BuOH treatment (3 h, 100°C) of samples (Reed *et al.*, 1982) Carob pod condensed tannins, supplied by Nestlé SA, were used as standard. This material was selected as standard because its anthocyanidin solutions have similar UV/VIS spectra as the grape samples (Fig. 2).

Standard lignin and Klason lignin were solubilized in HCl-activated triethyleneglycol at 125°C for 1 h. (Edwards, 1973). Total nitrogen was determined in the residues by the Kjeldahl method. Protein was calculated as $N \times 6{\cdot}25$.

Spectrophotometric determination of protein by the Biuret method (sandard: albumin; absorbances: 540 nm) and condensed tannins as anthocyanidin (Reed *et al.*, 1982), were carried out with the supernatant. The lignin solubilized was measured by the Morrison procedure (1972), reading the absorbance at 280 nm. Different amounts of lignin standard dissolved under the same conditions were used to determine the standard curve; corrections corresponding to the absorption of anthocyanidins were made. UV/VIS absorption spectra of lignin, standard CT anthocyanidins, WGP and RGP anthocyanidins and Biuret tests were performed.

RESULTS AND DISCUSSION

The results of the DF determinations in white grape pomace (WGP) and red grape pomace (RGP) are shown in Table 1. TDF values were similar for both samples.

The retention in DF residues of high percentages of protein in vegetables with appreciable amounts of condensed tannins (CT) after enzymatic treatments was reported in other samples. Moreover, protein–tannin complexes formation and protease inhibition prevent the removal of protein (Tamir & Alumot, 1969; Griffiths & Moseley, 1980; Kumar & Singh, 1984; Saura-Calixto, 1988). The protein found in DF residues of RGP and WGP represented 88·4 and 70·1%, respectively, of the total protein content in the original material (13·8 and 10·7% dry matter).

TABLE 1
Dietary Fibre Content of Grape Pomaces (% dry matter)

	Red grape pomace	*White grape pomace*
Insoluble dietary fibre	65·7 ± 0·6	62·5 ± 1·1
Protein in insoluble dietary fibre	12·2 ± 0·3	7·5 ± 0·2
Insoluble dietary fibre (corrected value)	53·5	55·0
Soluble dietary fibre	7·3 ± 0·4	4·0 ± 0·3
Total dietary fibre (AOAC method)	60·8	59·0
Total dietary fibre (including condensed tannins and indigestible protein)	73·0	66·5
Condensed tannins	36·4 ± 0·9	14·5 ± 0·5

Mean value of five determinations ± standard deviation.

Famuyiwa and Ough (1982) reported low digestibilities of several grape pomaces with different phenolic contents. The lowest digestibility corresponded to the highest total phenol content. The protein binding properties of condensed tannins from grape pomaces immobilized on Sepharose 4B were similar to that from tannins in free solution (Oh *et al.*, 1985). Reed *et al.* (1982), found CT and crude protein in the neutral detergent fibre from Cassava; the amount of insoluble protein they found in this forage on heating with pepsin or protease correlated very highly with the amount of CT.

Two DF values are given in Table 1. The total DF values by the AOAC method correspond to the definition of DF as 'NSP and lignin', and were obtained by protein subtraction (Prosky *et al.*, 1988). Nevertheless, the CT contents in grape pomaces represent a major percentage of the DF residues. Although the AOAC methods consider the DF values to correspond to NSP and lignin, the DF values for grape pomaces contained CT as well as NSP and lignin.

The second set of the DF values in Table 1 (73·0 and 66·5%) includes CT and RP; that is to say, the substances that are not digested by the enzymatic treatments.

The contents of the main DF constituents (KL, neutral and acidic polysaccharides) are listed in Table 2. The values of DF obtained from the sums of these compounds, after the subtraction of protein in KL, accord with the results obtained with the AOAC method. The small differences (RGP – IDF, 53·5 – 51·7 = 1·8; VGP – IDF, 55·0 – 53·7 = 1·3) can be due

TABLE 2
Klason Lignin (KL) and Non-Starch Polysaccharides (NSP) of Grape Pomaces. (% dry matter)

	Red grape pomace			*White grape pomace*		
	IDF	*TDF*	*SDF*	*IDF*	*TDF*	*SDF*
Neutral sugars	11·7 ± 0·3	15·8 ± 0·3	4·1	12·5 ± 0·2	13·5 ± 0·3	1·0
Uronic acids	3·1 ± 0·1	7·0 ± 0·2	3·9	2·6 ± 0·1	3·9 ± 0·1	1·3
NSP	14·8	22·8	8·0	15·1	17·4	2·3
KL	39·7 ± 0·6	39·7		41·2 ± 1·2	41·2	
NSP plus KL	54·5	62·5	8·0	56·3	58·6	2·3
Protein in KL	2·8 ± 0·05			2·6 ± 0·1		
NSP plus KL (corrected value)	51·7			53·7		

Mean value of three determinations + standard deviation.
SDF (Soluble Dietary Fibre) = TDF (Total Dietary Fibre) − IDF (Insoluble Dietary Fibre).

to a partial removal of CT during the KL acid treatment. To check this, the KL procedure was performed on CT standard; 7·43 ± 0·30% CT was found in the supernatant.

Table 3 gives the results of the determinations of lignin, CT and protein in the supernatants obtained by HCl/triethyleneglycol treatment of KL residues. This treatment only dissolved part of the lignin and condensed tannins in the original residues, while the lignin standard was completely dissolved by the same treatment. This finding may indicate the strength of the lignin–tannin–protein links.

The fact that protein was not detected in the supernatant can be confirmed by comparing the spectra of the Biuret test solutions (Fig. 3); the spectrum for WGP does not have the λ_{max} at 540 nm as in the albumin standard. On the other hand, the spectra for the grape pomace supernatants (i.e., spectrum 3, Fig. 3) have two λ_{max} in the ultraviolet and visible regions, corresponding to the partial solutions of lignin and anthocyanidins, respectively.

TABLE 3
Substances Solubilised by HCl/Triethylene Glycol Treatment of Klason Lignin. (% original dry pomace)

	Red grape pomace	*White grape pomace*
Lignin	2·6 ± 0·1	2·5 ± 0·1
Condensed tannins	7·6 ± 0·1	2·2 ± 0·05
Protein	Not detected	Not detected

Mean value of three determinations ± standard deviation.

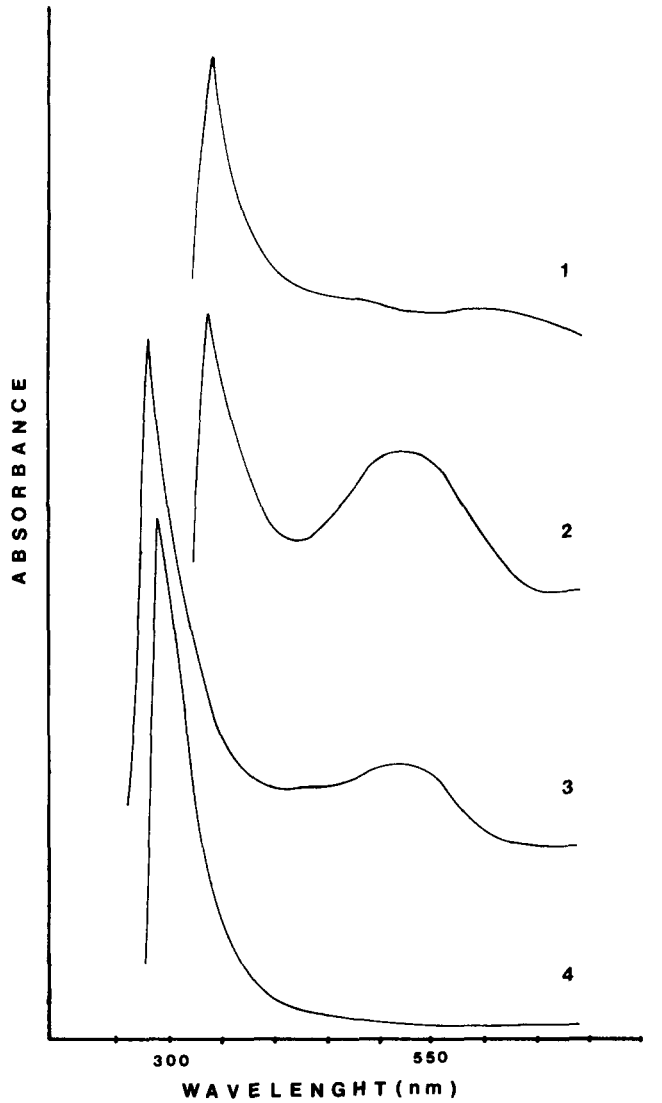

Fig. 3. UV/VIS absorption spectra of the supernatant obtained from KL of WGP and lignin standard by HCl/triethylene glycol treatment. Biuret test of white grape pomace supernatant (1); Biuret test of albumin standard (2); Supernatant of WGP (3); Supernatant of lignin standard (4).

The data of the protein found in IDF and KL residues for grape pomaces, are completed with the results corresponding to other samples previously reported (Table 4). The samples checked have appreciable amounts of CT and protein. An important part of the proteins remain in IDF residues after the enzymatic treatments (amylase, protease and amyloglucosidase). When these IDF residues are treated with sulfuric acid (12M, 20°C, 3 h; 1M, reflux,

TABLE 4
Protein in Insoluble Dietary Fibre (IDF) and Klason Lignin (KL) Residues for Samples Rich in Condensed Tannins (CT). (% dry matter of original sample)

	CT in sample	*Protein in sample*	*Protein in IDF*	*Protein in KL*
Carob pod[a]	17·9	2·7	1·8	0·8
Cider wastes[a]	3·1	5·4	3·0	1·9
Spanish sainfoin[a]	7·0	8·2	2·1	ND
White grape pomace	14·5	10·7	7·5	2·6
Red grape pomace	36·4	13·8	12·2	2·8

ND, not determined.
[a] From Saura-Calixto (1987; 1988) and Goñi *et al.* (1989).

2 h) the corresponding residues (Klason lignin) also contain significant amounts of protein. The protein from the KL residues obtained from grape pomaces is not even dissolved by the HCl/triethyleneglycol treatment (see Table 3).

These data clearly show the presence of indigestible protein–tannin complexes in IDF and KL residues. This seems to indicate that CT and resistant protein could be considered as DF components. Trowell (1988) has recently suggested the consideration of resistant protein as a DF constituent.

The DF values corresponding to NSP plus lignin for samples containing CT may be less predictive of physiological function than the values including CT and RP because these compounds are strongly bound to the polysaccharide matrix and escape digestion. The actual indigestible residue will be appreciably higher than the NSP plus lignin content.

Both fractions (NSP–lignin and CT–RP) will contribute to some typical physiological properties of DF (water holding capacity, binding of minerals and bile acids retardation of intestinal glucose absorption, etc.). Additionally, CT could lower the starch digestion because of the α-amylase inhibition.

'Indigestible polysaccharides, phenolic polymers and resistant protein' could be considered as a wider definition of the DF complex. The term 'phenolic polymers' includes both CT and lignin (polymer of phenylpropane units). A certain proportion of the plant protein of the vegetables is not digested and, what is more, CT are common in the diet (grapes, apples, cocoa, pear, plum, sorghum, kiwi, strawberry, blackberry, peach, legumes, etc.).

Existing DF methodology provides suitable procedures to determine indigestible polysaccharides (NSP and resistant starch) although further research is needed to find simpler methods for determination of resistant proteins and phenolic polymers in vegetables and fruits.

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