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Nature of the condensed tannins present in the dietary fibre fractions in foods

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Condensed tannins may be regarded as indigestible, or at least of only limited digestibility, throughout their presence in the dietary fibre fractions of different foodstuffs. This study examines the condensed tannins in the soluble and insoluble dietary fibre fractions of three quite different foods: pears, lentils and cocoa. Mean molecule size of the tannins present in the dietary fibre fractions was larger than that of the tannins present in the foodstuffs overall. The protein present in both the whole foodstuffs and in the dietary fibre fractions alone was also studied, and relationships between protein content and condensed tannin size were established.

INTRODUCTION

Condensed tannins (CTs) or polymers of proanthocyanidins, are phenolic compounds that are widely present in vegetables (Metche, 1980). They consist of flavan-3-ol units with C_4–C_8, C_4–C_6 and C_2–C_7 bonds.

Because of their ability to form complexes with proteins, they are directly responsible for the astringency of foods that are rich in such compounds (Haslam & Lilley, 1988).

In-vivo experiments carried out in animals have shown that diets high in CTs tend to bring about such negative nutritional effects as reduced appetite, lower nutrient utilisation, and slower growth rates (Reddy *et al.*, 1985, Butler, 1989; Clausen *et al.*, 1990; Salunke *et al.*, 1990), though final results may vary according to the animal species studied (Singleton, 1981). From a physiological standpoint, CTs appear not to be absorbed by the digestive tract (Mitjavila, 1980; Jiménez-Ramsey *et al.*, 1994) and to be excreted almost in their entirety (Bravo *et al.*, 1992; Jiménez-Ramsey *et al.*, 1994).

In view of the difficulties involved in carrying out such experiments on human beings, the interactions between CTs and different nutrients (carbohydrates, vitamins, alkaloids, metals, proteins, etc.) have been analysed and measured in model systems, with proteins being the most important. Protein size, conformation, charge and the amount of proline present in the protein (Hagerman & Butler, 1981) as well as tannin composition and conformation (Asquith & Butler, 1986; Ricardo da Silva *et al.*, 1991) all influence tannin–protein interactions.

New in vitro experiments that simulate human digestion and take into account the food matrix in which the CTs are found need to be designed to further the study of CTs in human nutrition and determine the importance of tannin–protein interactions.

The dietary fibre fraction in a foodstuff is the part of that foodstuff regarded as indigestible or resistant to the action of human digestive enzymes (Trowell, 1974; Van Soest, 1984; Hunt & Groff, 1990; Saura-Calixto *et al.*, 1991). Studying the CTs in this food fraction will help to determine the degree of digestibility of CTs and the variables that may affect such digestibility (CT content and composition of the whole food). The object of the present study was to characterise and quantify the CTs present in the soluble and insoluble dietary fibre fractions of several foodstuffs. To this end, different methods for analysing phenolic compounds were employed, and different mathematical relationships between the analytical values were established. The proline content and the proline richness of the proteins

in the two dietary fibre fractions and in the whole food were assessed with a view to determining the possible interactions between CTs and proteins.

MATERIALS AND METHODS

Sample selection and preparation

In an endeavour to make this study as general as possible, three foodstuffs with quite different nutritional characteristics were chosen: pears (*Pyrus communis*), high in sugars and low in protein and fat; lentils (*Lens esculenta*), high in protein and low in fat, and cocoa (*Theobroma cacao*) beans, high in fat and polyphenols and low in protein.

The juice of the pear samples was discarded. A de-juicer was used to produce a solid residue, which was dried in an oven at 60°C for 24h. After drying, samples were comminuted to a particle size of less than 0·5mm.

The lentil samples were likewise comminuted to a particle size smaller than 0·5mm.

The fat was extracted from the raw cocoa beans using petroleum ether, after which the samples were dried in the same manner as the pear samples and comminuted as above.

This dried and comminuted material from each type of foodstuff is considered as the whole foodstuff and shall hereinafter be referred to as 'dry material'.

Dietary fibre fractions

The enzymatic-gravimetric method of the AOAC for the determination of dietary fibre (Prosky *et al.*, 1988) is based on digestion of samples by α-amylase, protease, and amyloglucosidase (Fig. 1). This procedure yields two indigestible residues: the insoluble dietary fibre fraction (IDFF), namely, the solid material resistant to this digestion process; and the soluble dietary fibre fraction (SDFF), namely, the material precipitated by adding 95% ethanol to the supernatant liquid obtained by filtering out the IDFF (80:20 v/v).

Extraction of phenolic compounds

The phenolic compounds were extracted using acetone/water (70:30, v/v) at 4°C. An amount of 0·5g of sample and 10ml of extractant were mixed in a 15ml test tube and shaken for 20min, after which time the extract was separated by centrifugation (2000 rpm for 10min). The extract was used in the subsequent analyses.

Total polyphenols

The Prussian blue method (Price & Butler, 1977), which interferes with proteins less than such other oxidation–reduction reactions as the Folin–Ciocalteus method (Hagerman & Butler, 1989) was employed. The results have been expressed as tannic acid (Sigma Chemical Co.).

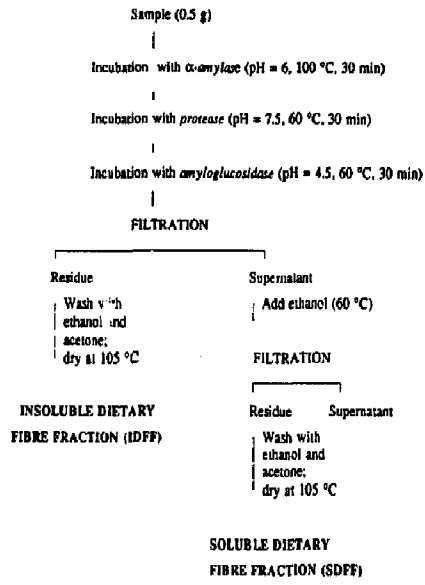

Fig. 1. Preparation of the dietary fibre fractions.

Condensed tannins

A modified version of the method of Porter *et al.* (1986) for hydrolysis with butanol/HCl using polyvinylpyrrolidone as a phenol adsorber (Watterson & Butler, 1983) was employed. This modification enabled all the food samples to be assayed and averted possible interference by the various sugars (abundant in the pears) in the colour reactions measured at 550 nm. There is no universal reference standard for condensed tannins that could be used for comparing the results. Quebracho tannins (Asquith & Butler, 1985) were used as the standard in this study.

Relative degree of polymerisation (relative chain length)

The ratio between the absorbance measurement value obtained using the method of hydrolysis with butanol/HCl and the value obtained using the method of reaction with vanillin in acetic acid (Butler *et al.*, 1982) was used (relative chain length).

Protein and proline analysis

The protein content of the samples was calculated as the difference between the measurements for total amino acids (obtained using hydrolysed sample) and for the free amino acids (obtained using unhydrolysed sample) (Mole *et al.*, 1990). Amino acid analysis was performed according to the method of Mole and Waterman (1985) using ninhydrin as reagent. The results have been expressed as L-leucine (Sigma Chemical Co.).

The proline content of the proteins has been expressed as the difference between the proline measurement value for hydrolysed sample and that same value for unhydrolysed sample (free proline) (Mole *et al.*, 1990). The method of Bates *et al.* (1973) was employed for proline analysis. The results have been expressed as L-proline (Sigma Chemical Co.).

Hydrolysis was carried out by placing 0·1 g of sample and 8 ml of 6M HCl in a closed test tube at 100°C for 8 h, after which the mixture was then partially neutralised with 4 ml of 1M NaOH. For unhydrolysed sample, 0·1 g of sample was mixed with 12 ml of NaCl solution for the same amount of time, but at ambient temperature. The supernatants of each mixture obtained by filtration were used in the analyses.

RESULTS AND DISCUSSION

Of the three foodstuffs considered, lentils had the lowest total dietary fibre content (Table 1, %TDFF) and hence can be regarded as the most highly digestible, as opposed to cocoa and pear, in which dietary fibre accounted for approximately half the weight of the food, though the percentage proportions of the soluble and insoluble dietary fibre fractions in these two foodstuffs differed (Table 1, %IDFF/%SDFF).

Table 1. Soluble and insoluble dietary fibre fractions[a] (expressed as % dry material)

	%SDFF	%IDFF	%TDFF	%IDFF/ %SDFF
Pears	11·24 ± 0·43	45·59 ± 1·37	56·83	4·05
Lentils	6·71 ± 0·60	20·24 ± 0·90	26·95	3·01
Cacao beans	8·74 ± 0·61	48·97 ± 1·47	57·71	5·60

[a]Three replications of all determinations were performed. SDFF — Soluble dietary fibre fraction. IDFF — Insoluble dietary fibre fraction. TDFF — Total dietary fibre fraction.

Cocoa had the highest total phenol content (Table 2, total phenols); lentils had an intermediate total phenol content, whereas the total phenol content in the pears was low. The phenol content in the soluble and insoluble indigestible fractions was lower than in the whole food (dry material). The values for the soluble and insoluble dietary fibre fractions varied in each of the foodstuffs considered; in the pears and lentils the two fractions presented quite similar total phenol values, whereas in the cocoa the phenol content of the insoluble fraction was nearly 10 times higher than that of the soluble fraction.

The condensed tannin values (Table 2, condensed tannins) in the different samples followed the same pattern as the total phenols.

In those cases in which the degree of tannin polymerisation could be calculated (Table 2, relative chain length), relative chain length for the fibre fractions was longer than in the whole food (dry material). This indicates that the tannins present in the fibre fractions differ from those present in the whole food at least with respect to size.

The ratio of condensed tannins to total phenols (Table 2, condensed tannins/total phenols) was highest in the lentils and lowest in the pears. The intermediate value recorded for the cocoa, despite that foodstuff's high tannin content, is indicative of the importance of the anthocyanins in these samples (Mazza & Miniati, 1993). A similar explanation may account for the results obtained for the pears, possibly caused by the presence of mainly low-molecular-weight phenols such

Table 2. Results of polyphenol analyses[a]

Sample	Total phenols[b]	Condensed tannins[c]	Flavan-3-ol end[d]	Relative chain length[e]	Condensed tannins[c]/total phenols[b]	CAP[f]	CAP[f]/total phenols[b]
Pears							
Dry material	3·35 ± 0·10	0·56 ± 0·02	0·12 ± 0·02	1·56	0·17	0·87	0·26
SDFF	1·86 ± 0·15	0·27 ± 0·02	0·03 ± 0·01	2·00	0·15	0·54	0·29
IDFF	1·59 ± 0·14	ND[g]	T[h]	—	—	—	—
Lentils							
Dry material	7·76 ± 0·50	1·92 ± 0·17	1·17 ± 0·05	0·72	0·25	1·38	0·18
SDFF	3·39 ± 0·12	0·48 ± 0·03	0·15 ± 0·01	1·00	0·14	0·48	0·14
IDFF	2·67 ± 0·13	0·52 ± 0·04	0·10 ± 0·01	1·75	0·20	0·91	0·34
Cocoa							
Dry material	58·81 ± 1·19	11·46 ± 0·46	11·27 ± 0·60	0·48	0·20	5·50	0·10
SDFF	1·95 ± 0·16	0·32 ± 0·03	0·12 ± 0·02	1·55	0·16	0·50	0·25
IDFF	19·12 ± 1·32	4·43 ± 0·21	1·85 ± 0·15	1·11	0·23	4·92	0·26

[a]Three replications of all determinations were performed. SDFF — Soluble dietary fibre fraction. IDFF — Insoluble dietary fibre fraction.
[b]Prussian blue assay, mg tannic acid/g sample.
[c]Butanol/HCl hydrolysis, mg quebracho tannins/g sample.
[d]Vanillin in acetic acid, mg catechin/g sample.
[e]See text.
[f]CAP is defined as condensed tannins × relative chain length (see text).
[g]ND — Not detected.
[h]T — Under 0·02.

as hydroxycinnamic acids and flavonols (Macheix *et al.*, 1990). The values for this ratio were higher for the insoluble dietary fibre fraction than for the corresponding soluble dietary fibre fraction in the foods in which measurements enabled this ratio to be calculated.

As was expected, the lentils presented the highest protein content, followed by the cocoa and the pears (Table 3, protein). The residual protein value for the lentils was lower than that for the cocoa and the pears (Table 3, residual protein), indicating that the proteins in the lentils were more digestible.

The pattern for the proline richness of the proteins (Table 3, proline richness of the protein) was different among the three foodstuffs studied. Cocoa had the highest values, followed by the lentils and then the pears. This indicates the different compositions of the proteins in each of the foodstuffs analysed.

The values for the proline richness of the proteins in the insoluble dietary fibre fractions (Table 3, proline richness of the protein) were higher than those for the dry material in the cocoa and the lentils, but not in the pears, in which the percentage protein richness value for the insoluble dietary fibre fraction, though similar, was lower. Viewed in the light of the CT results, the tannin–protein interaction would appear to be partly responsible for these findings, because the CTs were not detectable in the insoluble dietary fibre fraction in the pears.

On the other hand, the values for the proline richness of the proteins in the soluble dietary fibre fractions were quite similar in the three foodstuffs considered here, indicating that the behaviour of this fraction is unlike that of the insoluble dietary fibre fraction. This fraction was obtained by precipitation of the supernatant from enzymatic digestion of the dry material in an alcohol medium (Fig. 1); hence the finding of a higher degree of similarity between the soluble fractions of the three foodstuffs than between the insoluble fractions seems reasonable, inasmuch as the composition of the soluble dietary fibre fraction was more dependent upon the action of the enzyme systems than on the original foodstuff itself.

It has been reported that the CT content of the neutral dietary fibre fraction of certain feeds is negatively correlated with protein degradability (Rittner & Reed, 1992). This may also be inferred from the results obtained for CT and residual protein, for the insoluble fibre fractions (Table 2, condensed tannins, and Table 3, residual protein) showed these two values to be related in the cocoa and the lentils, in that higher CT values were associated with higher residual protein values, i.e. lower protein degradability. However, bearing in mind that the insoluble fibre is the solid residue after digestion, the composition of that fraction is highly dependent upon the initial foodstuff matrix, and hence it may be venturesome to attempt to draw definite conclusions for such widely different foods.

In contrast, since the composition of the soluble dietary fibre is less dependent upon the initial foodstuff matrix, a relationship between the CT content and the percentage residual protein could be established for the soluble fibre fractions and this confirmed the tannin–protein interaction. The highest CT value, in the lentils, was associated with the lowest residual protein value, the converse of what might be expected; however, the CTs of the soluble dietary fibre fraction in the lentils presented the lowest relative mean size (chain length 1·00 as opposed to 2·00 in the pears and 1·55 in the cocoa). This interaction depends not only upon quantitative variables but also upon such qualitative variables as protein size, composition, and shape and tannin size and structure.

Table 3. Protein and protein proline contents[a]

Sample	Protein[b]	Residual protein[c] (%)	Protein proline[d]	Residual protein proline[e] (%)	Proline richness of the protein[f]
Pears					
Dry material	2·81 ± 0·20		0·11 ± 0·01		3·91
SDFF	6·60 ± 0·21	26·4	0·36 ± 0·02	36·8	5·45
IDFF	3·84 ± 0·20	62·3	0·14 ± 0·01	58·0	3·65
Lentils					
Dry material	21·0 ± 0·89		1·10 ± 0·04		5·25
SDFF	13·6 ± 0·35	4·35	0·80 ± 0·01	5·80	5·90
IDFF	16·2 ± 0·70	15·6	1·08 ± 0·04	19·9	6·67
Cocoa					
Dry material	11·9 ± 0·45		0·89 ± 0·08		7·50
SDFF	9·30 ± 0·18	6·85	0·56 ± 0·03	5·50	6·02
IDFF	13·8 ± 0·33	56·9	1·10 ± 0·03	60·5	7·98

[a]Three replications of all determinations were performed. SDFF — Soluble dietary fibre fraction. IDFF — Insoluble dietary fibre fraction.
[b]Protein content, g L-leucine/100 g sample.
[c]%Protein (dry material) in DF fractions. Calculated as (Protein (SDFF)[b] × %SDFF)/(protein (dry material))[b]. By way of example, the residual protein in the pear SDFF is (6·60 × 11·2)/2·81 = 26·4.
[d]Protein proline content, g L-proline/100 g sample.
[e]Protein proline (dry material) in DF fractions. Calculated as (Protein proline (SDFF)[d] × %SDFF). By way of example, the residual protein in the pear SDFF is 0·36 × 11·2/0·11 = 36·8.
[f]Protein proline in protein. Calculated as (Protein proline[d] × 100)/protein[b].

Since, except in highly specific instances, CT analysis by hydrolysis with butanol/HCl measures flavan-3-ol units irrespective of CT size and structure, it was considered appropriate to define tannin capacity (CAP) as a measure of the CT content that takes CT size into account. CAP is the product of multiplying the CT value obtained by hydrolysis using butanol/HCl by mean relative CT size: CAP = condensed tannins × relative chain length (Table 2, CAP). This is a mathematical contrivance to correct the measurement value based on flavan-3-ol units by taking into account the degree of polymerisation of the tannins such that, for a given CT value, the tannin capacity will be higher for higher degrees of tannin polymerisation.

Putting into the context of results for the soluble dietary fibre fraction, the CAP value for the lentils was slightly lower than that for the cocoa, and both these CAP values were lower than that for the pears (Table 2, CAP). This was the same order as in the case of the residual protein (Table 3, residual protein) though, in that case, the value for the soluble dietary fibre fraction in the pears was considerably higher than the values for the soluble dietary fibre fractions in the other two foodstuffs and may have been influenced by the very low protein content of the dry material. The results suggest that the higher residual protein content in the soluble dietary fibre fractions may have been caused by the tannin–protein interaction taking place in in-vitro digestion of a foodstuff.

The CAP to total phenols ratio was also calculated. Tannin capacity per unit of polyphenol was higher for the insoluble fibre fraction than for the dry material in both the lentils and the cocoa. Accordingly, enzymatic digestion brought about an increase in the tannin capacity in proportion to the total phenol content in the resulting residue. These results demonstrate that tannin size is a very important factor in studies of tannin solubility and digestion.

Based on the results described, the study of a large number of varieties of a single foodstuff is required to provide statistical confirmation.

CONCLUSIONS

Because condensed tannins are present in the dietary fibre fractions of different foods, they may be regarded as either indigestible or poorly digestible substances. In addition, the mean size of the CTs in the dietary fibre fractions in the three foodstuffs considered here was larger than the mean size of the CTs in the original foods, which suggests that the more highly polymerised CTs are more resistant to digestion.

The analysis of the protein fractions showed that (a) the proline content of the residual protein in the insoluble dietary fibre fractions was richer than that of the proteins in the whole food when initial CT levels in the food were high; and (b) the CT content in the soluble dietary fibre fraction may be correlated with the residual protein value. These results may be explained in terms of tannin–protein interactions, which have been considered in depth in the literature.

Finally, the parameter CAP may be very useful in studies on CTs in both in-vivo and in-vitro experiments.

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