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Extraction, Determination and Fractionation of Sorghum Polyphenols

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ABSTRACT

Interactions between variety, dehulling, milling, extraction and method of separation of sorghum polyphenols were studied. The results indicate that the sorghum variety free of red–brown coloured testa (e.g. Egyptian varieties) had a lower content of polyphenols compared with red-coloured varieties (e.g. the bird-resistant varieties). Most of the polyphenol content of the first varieties could be extracted either with methanol or 80% aqueous acetone and could be determined by Folin Ciocalteau (FC) and/or Ferric Ammonium Citrate (FAC) methods. The best solvents to extract, and the best methods to determine, the polyphenols of the bird-resistant varieties were (75%) dimethyl formamide or 80% aqueous acetone and the FAC or vanillin (V) methods, respectively. To get a good separation of sorghum polyphenols by TLC the following conditions were required: methanol as extractant, n-butanol:acetic acid:water (4:1:2 v/v/v) as a developing solvent and iodine vapour as visualizing reagent. Dehulling of sorghum grains decreased the polyphenol content while milling removed some of its fractions.

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INTRODUCTION

The presence of polyphenols in sorghum grain provides agronomic advantages such as resistance to bird depredation (McMillian *et al.*, 1972) and to preharvest seed mould (Harris & Burns, 1973). On the other hand, polyphenols bind certain proteins strongly and thus diminish the digestibility and nutritional value of sorghum grain (Price *et al.*, 1980).

The polyphenolic compounds in sorghum include mainly the flavonoids, anthocyanidin, flavanols (tannins) and phenolic acids (Hahn *et al.*, 1984). According to Hoseney *et al.* (1981) sorghum grains are free of hydrolyzable tannins. However, the condensed tannins (proanthocyanidin 'polymer of hydroxy flavon 3 ol', catechin) are present in some sorghum varieties (Butler, 1982). Due to the low concentrations and problems of isolation, the chemical nature of the sorghum polyphenols is ill-defined (King, 1962).

The extraction, determination and fractionation of the polyphenols of the Egyptian sorghum varieties compared with a high tannin foreign variety were investigated in this study. The Folin Ciocalteau (FC) and Prussian Blue (PB) procedures were used to determine total polyphenols, while vanillin (V) and Ferric Ammonium Sulphate (FAC) methods were used for estimating flavanols (tannins) or polyphenols. Iodine Vapour (IV) was also tried in an attempt to detect flavanoids.

MATERIALS AND METHODS

Materials

Whole grains

Three sorghum grains belonging to the species *Sorghum vulgare* were utilized in this study. The first variety was from the bird-resistant sorghum group 'BR' (with a pigmented testa layer). It was obtained in the summer of 1983 from Lippische, Hauptgenossenschaft Company, Detmold, West Germany. The other two varieties, Giza 15 and NES 1007 (free of pigmented testa) were obtained in the summer of 1984, from the Ministry of Agriculture, Agricultural Research Centre, Cairo, Egypt. Also, wheat grain (strong type variety CWRS) was obtained from Bundesforschungsanstalt für Getreide und Kartoffelvorarbeitung, Detmold, West Germany.

Dehulled grains

The sorghum grains were dehulled using a vertical shelling machine type '270'.

Flours

Both sorghum and wheat grains were milled using a Buhler Automatic. Laboratory Mill type '20', West Germany. The extraction rate was 82% for wheat and 70% for sorghum.

Methods

Preparation of samples

Samples of whole and dehulled grains were ground to pass through 60 mesh sieve using a Junke Kunkel, type A 10, electric blender. The ground samples were defatted by shaking with petroleum ether (40–60°C) at a ratio of 1:10 for 10 min at room temperature.

Polyphenol extraction

The polyphenol compounds of the defatted samples were extracted with each of the following solvents: methanol, acidic methanol (1% HCl), 80% aqueous acetone and 75% dimethylformamide (DMF) as described by Gupta & Haslam (1980).

Polyphenol determination

Four different methods; namely, Ferric Ammonium Citrate 'FAC' (Daiber, 1975), Prussian Blue 'PB' (Price & Butler, 1977), Folin Ciocalteau 'FC' (McGrath *et al.*, 1982) and Vanillin 'V' (Price *et al.*, 1978) were used to determine the polyphenols in whole and dehulled sorghum grains as well as their flours. Catechin was used as a standard for each of the above methods. The mean of triplicate measurements was expressed as catechin equivalents 'CE' g/100 g sample.

Polyphenol fractionation

TLC was used to fractionate the polyphenols extracted from whole, dehulled sorghum grains and the flour from such grains. In all cases, silica gel (Merek G, 60 mesh) was used for preparing a thin layer, 250 μ thick, on a glass plate of 20 × 20 cm. The developing solvent system suggested by Markham (1975) (*n*-butanol:acetic acid:water, 4:1:2 v/v/v) was used. The following visualization reagents were utilized: FC, V and iodine vapour (IV) (Engelshowe, 1976).

RESULTS AND DISCUSSION

Extraction and determination

From the results of polyphenol extraction and determination (Table 1), the following points could be concluded:

TABLE 1

Effect of the Extractant and the Method of Determination on the Polyphenols Content in Sorghum Grains (as Catechin Equivalents in g/100 g Sample, on Dry Weight Basis)

Variety/Determination method	*Extractant*											
	Methanol			*Acidic methanol (1% HCl in methanol)*			*80% acetone*			*75% DMF*		
	Whole grains (%)	*Dehulled grains (%)*	*Dehulling effect (%)*	*Whole grains (%)*	*Dehulled grains (%)*	*Dehulling effect (%)*	*Whole grains (%)*	*Dehulled grains (%)*	*Dehulling effect (%)*	*Whole grains (%)*	*Dehulled grains (%)*	*Dehulling effect (%)*
BR												
Ferric ammonium citrate	0·52	0·02	96·1	0·33	0·016	95·2	0·70	0·044	93·0	1·2	0·092	92·3
Prussian Blue	0·17	0·026	84·7	0·23	0·035	84·8	0·22	0·04	80·0	3·5	0·049	86·0
Folin Ciocalteau	0·35	0·049	86·0	0·27	0·04	85·2	0·34	0·052	81·8	3·7	0·055	85·1
Vanillin	2·9	0·100	96·5	3·4	0·12	96·5	3·5	0·13	96·3	3·9	0·17	95·6
Giza-15												
Ferric ammonium citrate	0·027	0·005	81·5	0·02	0·04	80·0	0·053	0·008	83	0·055	0·013	76·4
Prussian Blue	0·069	0·020	71·0	0·083	0·02	68·7	0·08	0·023	71·2	0·098	0·03	69·4
Folin Ciocalteau	0·082	0·025	69·5	0·066	0·02	69·7	0·08	0·023	71·2	0·10	0·031	69·0
Vanillin	0·016	0·002	87·5	0·020	0·003	85	0·018	0·003	83·3	0·025	0·004	84·0
NES-1007												
Ferric ammonium citrate	0·029	0·006	79·3	0·022	0·004	81·8	0·06	0·01	83·3	0·080	0·02	75·0
Prussian Blue	0·075	0·022	70·7	0·086	0·026	69·8	0·098	0·028	70·0	0·135	0·036	73·0
Folin Ciocaleau	0·11	0·033	68·2	0·074	0·021	71·6	0·102	0·030	70·5	0·130	0·036	72·0
Vanillin	0·025	0·004	84·0	0·028	0·004	85·7	0·025	0·004	84·0	0·033	0·006	81·8

(1) The polyphenol content varied according to variety, extractant and method of determination.

(2) The highest amount of polyphenol of the three sorghum varieties was extracted by 75% aqueous DMF, followed by 80% aqueous acetone and methanol. Acidification of methanol before extraction increased the value of polyphenols when determined by V and PB methods and lowered its content when determined by FAC and FC methods. The explanation for the differences in the extractability of the polyphenols is not yet known. It may be due to:
   (a) The occurrence of an acid-labile bond, such as a glycoside or an ester, which binds the tannins of sorghum to an insoluble compound of the grains.
   (b) The occurrence of an acid-labile structure which might limit the accessibility of the solvent to the polyphenols.
   (c) The structural characteristics of polyphenols which might require different solvents for extraction from the grains.

(3) In the BR sorghum variety the V method gave the highest polyphenol content. It was followed by FAC, FC and PB, respectively. In the case of the Egyptian varieties both PB and FC methods gave more polyphenol content (about 3–4 times more) than that found by FAC and V methods, respectively. Maxson & Rooney (1972) reported that:
   (a) The PB and V methods estimated different phenolic compounds in the sorghum grain.
   (b) The FAC method gave a low value for tannic acid equivalent.
   (c) The amount of condensed tannins determined as catechin equivalents with the V-HCl method was five to 15 times as high as the FAC and PB methods and differed 15-fold between the varieties.
   (d) The Folin reagent method was more sensitive to non-tannin phenolic compounds than either the FAC or V-methods.
   (e) The condensed tannins are the predominant type for tannins in sorghum.

(4) A marked reduction in polyphenol content occurred after the dehulling process of sorghum. This is an indication that most of the polyphenols are concentrated in the pericarp of the sorghum grains. This reduction ranged from 68% to 96·6% according to sorghum variety and determination method. Generally it was higher in the BR variety with a pigmented testa than the Egyptian varieties free of testa and also when both FAC and V methods were used as against PB and FC methods. Slight variations in this reduction were noticed among the solvents used for polyphenol extraction.

(5) According to the classification of Cummings & Axtell (1973) and Price *et al.* (1978) which is based on using the V method for polyphenol determination and the microscopic examination for testa detection, the BR variety belongs to sorghum group III (CE > 1% and with a coloured testa) while the Egyptian varieties belong to sorghum group I (CE < 1% and free from testa).

Fractionation and identification

Different trials were carried out to fractionate and to identify the polyphenols of sorghum grains using the TLC technique.

As shown in Table 2, when FC was used as a visualization reagent the following points could be deduced:

(1) The separated bands on the TLC plate differed in both numbers and Rf-values according to sorghum variety and polyphenol extractant.
(2) The number and Rf-values of the bands with each of polyphenol extractants were identical in the Egyptian sorghum varieties. The bands numbered 3, 5, 8 and 11 were found in all three sorghum varieties. The other bands varied between BR and the Egyptian varieties.
(3) Both methanol and 80% aqueous acetone extractants gave more separated polyphenolic compounds than H^+-methanol and aqueous DMF.

These results differed when the V reagent was used (Table 2). These variations were due to the specificity of the vanillin reagent for a narrow range of polyphenolic compounds such as catechin, leucoanthocyanidin and proanthocyanidin (Hahn *et al.*, 1984). The FC reagent is a general reagent to detect all the phenolic hydroxyl groups (McGrath *et al.*, 1982). Only bands Nos 6 and 11 of the Egyptian varieties and bands Nos 1, 5, 6, 8 and 11 in the BR variety from the compounds which consisted of flavon-3-ol units and/or tannins and the other bands in the three varieties are considered to be the simple phenolic compounds.

In the second trial, the effect of dehulling of sorghum on the removal of the polyphenols was studied. Dehulling of sorghum did not affect the number of bands separated but the intensities of these bands were lowered. This is an indication that the polyphenols were distributed in both hull and endosperm of the sorghum grain but in different concentrations.

Better resolution of the separated polyphenolic compounds in the crude methanol extract of the sorghum grains was obtained when IV was used as visualization reagent. It gave three bands more in the BR variety and one band more in the Egyptian varieties than the FC reagent. The Egyptian

TABLE 2
Numbers and Rf-Values of the TLC Bands of Sorghum Grain Polyphenols

Sorghum variety	*Band number*	*Polyphenol extractants*							
		Methanol		*Acidic Methanol (1% HCl)*		*80% Aqueous acetone*		*75% Aqueous DMF*	
		A	*B*	*A*	*B*	*A*	*B*	*A*	*B*
BR									
Base	1	0·00	0·00	—	0·00	0·00	0·00	0·00	0·00
	2	0·14	—	—	—	0·14	—	—	—
	3	0·21	—	0·21	—	0·21	—	0·21	—
	4	—	—	0·23	—	—	—	—	—
	5	0·33	—	0·33	—	0·33	—	0·33	—
	6	0·42	0·42	0·42	0·42	0·42	0·42	—	0·42
	7	—	0·46	0·46	0·46	—	0·46	—	0·46
	8	0·56	—	0·56	—	0·56	—	0·56	—
	9	0·63	0·63	—	—	—	0·63	0·63	0·63
	10	0·74	—	—	—	0·74	—	—	—
Front	11	0·86	0·84	0·86	—	0·86	0·84	0·86	—
Giza 15									
Base	1	0·00		—		0·00		0·00	
	2	0·14		—		0·14		—	
	3	0·21		—		0·21		—	
	4	—		—		0·23		0·23	
	5	0·33		0·33		0·33		0·33	
	6	0·42		0·42		0·42		0·42	
	7	—		0·46		—		—	
	8	0·56		0·56		0·56		0·56	
	9	0·63		—		0·63		—	
	10	0·74		0·74		0·74		0·74	
Front	11	0·86		0·86		0·86		0·86	
NES 1007									
Base									
	1	0·00	—	—	—	0·00	—	0·00	—
	2	0·14	—	—	—	0·14	—		
	3	0·21	—	—	—	0·21	—	—	—
	4	—	—	—	—	0·23	—	0·23	—
	5	0·33	—	0·33	—	0·33	—	0·33	—
	6	0·42	—	0·42	0·42	0·42	0·42	0·42	—
	7	—	—	0·46	—	—	—	—	—
	8	0·56	—	0·56	—	0·56	—	0·56	—
	9	0·63	—	—	—	0·63	—	—	—
	10	0·74	—	0·74	—	0·74	—	0·74	—
	11	0·86	0·84	0·84	—	0·84	0·84	0·84	—
Front									

A, Visualization by Folin Ciocalteau reagent; B, visualization by vanillin reagent.

sorghum varieties contained the same polyphenolic compounds as wheat grain.

Also the results reveal that milling removed the polyphenolic compounds located in the bran and germ of the sorghum grains. On the other hand, slight changes were noticed in both number and intensity of the separated polyphenolic compounds of wheat after milling. This means that most of these compounds are concentrated in wheat endosperm. Sorghum and wheat grains contained pelargonidin, catechin and phenolic acids, mainly in the endosperm of both grains.

In conclusion, the Egyptian sorghum varieties, which are free from testa, contained lower amounts of polyphenols, especially those that give positive reactions with V reagent ('Flavanols'), compared to the BR variety. The best solvent to extract, and the best method to determine, the polyphenols in the BR variety was 75% DMF or 80% aqueous acetone and the V or FAC methods while the 80% aqueous acetone (as extractant) and FC or FAC methods were preferred to determine the polyphenols in Egyptian varieties. Generally, polyphenols are distributed in different parts of sorghum grain. Dehulling and milling lowered polyphenol content particularly that concentrated in the bran and germ. The best conditions for fractionation of the total polyphenols by TLC were extraction with methanol, then *n*-butanol:acetic acid:H_2O (4:1:2 v/v/v) as developing solvent and iodine vapour as visualizing reagent.

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