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Tannin and non tannin-polyphenolic compounds of the Egyptian sorghum grains

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The fractionation and properties of the polyphenolic compounds of the Egyptian sorghum grains were studied. The results showed that sorghum grains contained a higher amount of non-tannin polyphenols than tannin ones. By utilizing the funnel separation technique higher recovery for both tannin and non-tannin polyphenol fractions was given compared with the Sephadex LH 20 column chromatography method. The refractionation of both fractions using T.L.C. technique showed that each fraction of tannin and non tannin composed of different compounds differed in their properties.

Introduction

Sorghum and barley contain undetected tannins [1]. HOSNEY et al. [2] found that tannins bind with most of the separation media used in chromatography. Thus, it is relatively easy to separate non-tannin polyphenolic compounds, phenolic acids and flavonoids from tannins chromatographically [3]. To the best of our knowledge nothing was known about the nature of the polyphenolic compounds in Egyptian sorghum varieties. Therefore in this article the fractionation of the polyphenolic compounds of the Egyptian sorghum grains into tannin and non-tannin compounds using different chromatographic technique was studied. The study also included one of the high tannin foreign sorghum varieties.

Materials and methods

Materials

Three sorghum varieties (*Sorghum vulgare*) were utilized in this study. The first variety belongs to the bird resistance sorghum group "BR". It was obtained from Lippische Hauptgenossenschaft Co., Detmold, FRG, in summer 1983. The other two varieties namely Giza 15 and NES 1007 were obtained from the Ministry of Agriculture, Cairo, Egypt, in summer 1984. Wheat grain of strong type, variety CWRS (W. Germany) was obtained from Milling Institute of Bundesforschungsanstalt für Getreide- und Kartoffelverarbeitung, Detmold, FRG.

Methods

Preparation of samples. Samples of whole sorghum grains were ground to pass through 60 mesh sieve using a Janke & Kunkel type A 10 electric blender. The ground samples were defatted by shaking with petroleum ether (40—60 °C) at ratio of 1:10 for 30 min at room temperature. The defatted samples were extracted

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with methanol to prepare the polyphenol extract according to the method of HULSE et al. [4]. The extracts obtained were concentrated under vacuum at 40 ° C using a rotary evaporator. Then it was weighed and referred to the total polyphenols.

Tannin and non-tannin polyphenols. The method described by MCGRATH et al. [3] to fractionate sorghum polyphenols into non-tannin (two fraction F_1 and F_2) and tannin (F_3 fraction) compounds was followed using Sephadex LH 20 (Pharmacia) column chromatography (5 × 100 cm) and 95% ethanol, 50% methanol and 50% acetone for elution. Each of the fractions obtained was concentrated under vacuum at 40 ° C using the rotary evaporator and reweighed. The procedure of ENGELSHOWE [5] to fractionate polyphenols into tannin (F_1) and non-tannin (F_2) fractions using a funnel separation technique was also utilized. Water was used to remove tannin and ethylacetate to remove non-tannin polyphenols. The weight of each fraction after drying under vacuum at 40 ° C by rotary evaporator were recorded.

Vanillin [6] and FOLIN-CIOCALTEU [3] (FC) methods were used to estimate the polyphenols content. The results are reported as catechin equivalent [g/100 g] in the crude extract, F_1 and F_2 fractions obtained by funnel separation. The solubility of these fractions in different solvents, colour (visually) and gelatin test [7] were also determined.

The fractions obtained from the previous methods were refractionated on T.L.C. glass plate (20 × 20 cm) coated with silica gel G 60 mesh (Merck) using the following developing solvent systems and visualizing reagents:

Developing solvent system

1. n-butanol:acetic acid:water (4:1:2 v/v/v) [5];
2. n-butanol:acetic acid:water (8:1:2 v/v/v) as suggested in this study;
3. Toluene:acetone:formic acid (30:30:6 v/v/v) [5].

Visualization reagent

1. Exposure to iodine vapour for flavonoid detection [8];
2. Spraying with FC reagent before heating for 10 min at 160 °C as a new trial during the present work for polyphenol detection;
3. Spraying with vanillin reagent (1% in methanol) then spraying with HCl (37%) followed by heating for 5 min at 150 °C for tannin or proanthocyanidin [5].

Results and discussion

Sephadex LH 20 column chromatography (C.C). The results of C.C. separation are reported in Table 1. The data in this table indicates that:

(1) Egyptian sorghum varieties contained higher contents of non-tannin polyphenols and lower content of tannin polyphenols compared with BR variety.

(2) Within non-tannin polyphenols, the low MW polyphenols F_1 (eluted with 95% ethanol) was relatively higher in BR variety than the Egyptian varieties. The opposite trend was noticed for F_2 fraction (eluted with 50% methanol).

In general the recovery of this procedure was only about 80%. Such decrease might be due to the ability of some polyphenols to bind with Sephadex or the lower ability of the eluted solution to recover all polyphenols. KING [9] found that some flavonoids of sorghum grains were bound to many chromatographic media.

The results of T.L.C. separation of the F_1, F_2 and F_3 fractions using n-butanol:acetic acid:water (4:1:2) as solvent system and either iodine vapour or FC as visulizing reagent reveal that:

(1) The number of the separated bands of fractions F_1 and F_2 were more in BR variety than in the two Egyptian varieties.

(2) Fraction three (F_3) gave one spot in the case of the BR variety and several bands in case of Egyptian varieties.

When visualization was carried out with vanillin reagent nothing was detected in F_1 and

Table 1
Yield of sephadex LH 20 column chromatography polyphenols fractions of sorghum grains

Sorghum variety/polyphenols	Yield [%]	
	A*	B**
BR		
Total polyphenols	2.4	100
Non-tannin polyphenols	1.4	58.4
— Fraction I (F_1)	1.18	49.2
— Fraction II (F_2)	0.22	9.2
Tannin polyphenols (F_3)	0.52	22.1
Recovery	1.93	80.5
Giza 15		
Total polyphenols	2.2	100
Non-tannin polyphenols	1.64	74.9
— Fraction I (F_1)	0.96	44.0
— Fraction II (F_2)	0.68	30.9
Tannin polyphenols (F_3)	0.13	5.9
Recovery	1.77	80.8
NES 1007		
Total polyphenols	2.2	100
Non-tannin polyphenols	1.63	73.1
— Fraction I (F_1)	0.91	40.9
— Fraction II (F_2)	0.72	32.2
Tannin polyphenols (F_3)	0.14	6.4
Recovery	1.77	79.5

* A = g extract/100 g ground sorghum grain;
** B = g eluate/100 g total polyphenols extract;
F_1 = eluate with 95% ethanol (low M. W non-tannin fraction);
F_2 = eluate with 50% ethanol (high M. W non-tannin fraction);
F_3 = eluate with 50% acetone (tannin fraction)

F_2 of the Egyptian varieties. This means that both fractions were free from tannin polyphenols.

T.L.C. separation was repeated for the three fractions using n-butanol:acetone:water (8:1:2 v/v/v) as a solvent system, the separated bands became more distinct and indicated that:

(1) Fraction one (F_1) was fractionated into 8 bands nearly identical in the three sorghum varieties but might be in various concentrations.

(2) Fraction two (F_2) was separated into 9, 11 and 12 bands in Giza 15, BR and NES 1007 respectively. The first 6 bands from the base line to the front of the T.L.C. plate were identical in the three sorghum varieties. The bands number 9 in Giza 15, 10 in NES 1007 and 12 in BR from the base line to the front were similar.

(3) Fraction three (F_3) was separated into 9 identical compounds in the Egyptian varieties and 5 compounds in BR variety. KALUZA et al. [10] used Sephadex and Sephacryl column chromatography to separate sorghum polyphenols. They obtained three fractions. Fraction number three was composed of two polymeric forms, one had approximately 1000 MW and the other was greater than 80000 MW.

Table 2
Yield, content and some properties of funnel separation tannin and non-tannin polyphenols of sorghum grains

Variety/Fraction	Yield [%]	Content as CE*				Solubility in					Colour	Gelatin test
		Vanillin method		FC method**		Methanol	Ethanol	DMF	Acetone	H_2O		
		A	B	A	B							
BR												
Total polyphenols	100	15.7		5.96		Soluble	Soluble	Soluble	Less soluble	Less soluble	Brownish red	Turbidity
Non-tannin polyphenols	63.3	8.5	34.3	3.2	34.0	Soluble	Soluble	Soluble	Less soluble	Less soluble	Brown	Turbidity
Tannin polyphenols	33.1	31.0	65.4	11.9	66.1	Soluble	Soluble	Soluble	Soluble	Soluble	Brownish	Precipitate
Recovery [%]	96.4											
Giza 15												
Total polyphenols	1.0	0.163		1.24		Soluble	Soluble	Soluble	Less soluble	Less soluble	Light orange	Turbidity
Non-tannin polyphenols	80.2	0.169	83.1	1.20	77.6	Soluble	Soluble	Soluble	Less soluble	Less soluble	Yellow	Turbidity
Tannin polyphenols	15.2	0.175	16.3	1.8	27.4	Soluble	Soluble	Soluble	Soluble	Soluble	Dark orange	Precipitate
Recovery [%]	95.4											
NES 1007												
Total polyphenols	100	0.224		1.92		Soluble	Soluble	Soluble	Less soluble	Less soluble	Dark orange	Turbidity
Non-tannin-polyphenols	75.0	0.236	79.0	1.78	69.5	Soluble	Soluble	Soluble	Less soluble	Less soluble	Orange	Turbidity
Tannin polyphenols	19.5	0.240	20.9	3.0	30.5	Soluble	Soluble	Soluble	Soluble	Soluble	Dark orange	Precipitate
Recovery [%]	94.5											

* CE — catechin equivalent; **FC — FOLIN-CIOCALTEU method;
A — g catechin/100 g;

$$\text{B — Percentage of total polyphenol content} = \frac{\text{yield [\%]} \times \text{polyphenols content [\%]}}{\text{total polyphenols [\%]}}$$

Funnel separation. As about 20% of the total polyphenols were lost when Sephadex LH 20 column chromatography was followed the funnel separation method was tried and its results in Table 2 reveal that:

(1) The recovery of the total polyphenols increased to 95%. This increase was 4.9, 5.3 and 2% in the non-tannin polyphenol fraction and 11, 9.3 and 13.1% in the yield of tannin polyphenol fraction of BR, Giza 15 and NES 1007 sorghum varieties respectively.

(2) The non-tannin and tannin polyphenols content as catechin equivalent [g/100 g] varied according to sorghum variety and method of determination. Generally most of the polyphenols in the Egyptian varieties are non-tannin polyphenols, while in BR variety are tannin. The FC method also gave higher value for tannin and non-tannin polyphenols as compared with vanillin procedure.

(3) Tannin polyphenols fraction was soluble in all tried solvents, had a colour ranging from dark orange in the Egyptian sorghum varieties to red brown in BR variety and precipitated gelatin.

(4) Non-tannin polyphenols fraction was less soluble in both water and acetone and soluble in the other tried solvents. Their colour was brown in BR, yellow in Giza 15 and orange in NES 1007 variety. They caused a turbidity for gelatin solution. Generally these properties except colour were identical to that of the crude methanol extract of sorghum grains.

When the crude methanol extract and both non-tannin and tannin polyphenols fractions of sorghum and wheat grains obtained by funnel separation technique were subjected to T.L.C. fractionation using n-butanol: acetic acid: water (4:1:2 v/v/v) as a developing solvent system and an iodine vapour for visualization. The results indicated that:

(1) Non-tannin polyphenols fraction was fractionated into 7 identical bands in the case of the Egyptian sorghum varieties. In BR variety 8 bands were separated, 7 of them were similar to those found in the Egyptian varieties. In wheat 10 bands were separated. Two of them were different from those found in BR sorghum variety.

(2) Tannin polyphenols fraction was fractionated into 2 distinct bands, one on the base line and the other with 0.3 Rf in BR and one spot with 0.2 Rf in the Egyptian sorghum varieties. In the case of wheat 2 fractions were identified far from the base line.

When T.L.C. was repeated using toluene: acetone: water (30:30:6 v/v/v) as a developing solvent system and an iodine vapour for visualization the non-tannin polyphenols fraction was fractionated into many different bands, while tannin polyphenols fraction of the three varieties appeared as one spot on the base line.

The previous results indicate that the Egyptian sorghum varieties contained a higher amount of non-tannin polyphenols than tannin one. Funnel separation technique gave higher recovery of tannin and non-tannin polyphenol than Sephadex LH 20 column chromatography. The three sorghum varieties contained nearly the same compounds of non-tannin polyphenols and differed mainly in their content of tannin polyphenols.

Zusammenfassung

A. M. M. YOUSSEF, Y. G. MOHARRAM, E. K. MOUSTAFA and H. BOLLING: Tannin und Nichttannin-Polyphenole in ägyptischem Sorghum

Es wurden die polyphenolischen Verbindungen von ägyptischem Sorghum fraktioniert und deren Eigenschaften untersucht. Die Untersuchungen zeigten, daß Sorghum mehr Nichttannin-Polyphenole enthält als Tannin. Durch Ausschütteln (Scheidetrichter) wurden größere Mengen an Tannin und Nichttannin-Poly-

phenolen gewonnen als mit der Sephadex LH 20-Säulenchromatographie. Die nochmalige Fraktionierung der beiden Fraktionen mit Hilfe der Dünnschichtchromatographie zeigte, daß sowohl die Tannin- als auch die Nichttannin-Fraktion aus unterschiedlichen Verbindungen zusammengesetzt sind, die sich in ihren Eigenschaften unterscheiden.

Резюме

А. М. М. Йоссеф, Й. Г. Мохаррам, Е. К. Моустафа и Х. Боллинг: Танин и нетаниновые полифенолы в египетском сорго

Фракционировались полифенольные соединения египетского сорго и исследовались их свойства. Исследования показали, что в сорго содержатся больше нетаниновых полифенолов чем танина. Путем взбалтывания (делительная воронка) получены большие количества танина и нетаниновых полифенолов чем колонковой хроматографией на сефадексе LH 20. Повторное фракционирование обеих фракций с помощью тонкослойной хроматографии показало, что как таниновая так и нетаниновые фракции состоят из разлизчных соединений, отличающихся по своим свойствам.

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