PHYTOCHEMICAL PROFILE AND NUTRACEUTICAL PROPERTIES OF DIFFERENT TYPES OF SORGHUMS

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INTRODUCTION

The high demand for antioxidant and nutraceutical foods has increased during the past years to prevent oxidative stress associated to the development of chronic diseases such as cardiovascular, neuron degeneration, cancer, diabetes and hypercholesterolemia as well as being involved with the process of aging (Grundy 2004, Wu et al., 2004). Recent research has demonstrated that the consumption of whole grains reduces the risk of these diseases due to the dietary fibre and phytochemicals, which are mainly concentrated in the bran. Their health benefits have been related to their high antioxidant and antiradical activities but also to many other mechanisms such as anti-mutagenic, estrogenic activities, inhibition of enzymes and induction of detoxification enzymes (Fimognari et al., 2004, Matsumoto et al., 2004). Sorghum [*Sorghum bicolor* (L.) Moench] is one of the most important grains in Africa and some parts of Asia, where it is consumed in many traditional foods (Rooney and Serna Saldivar, 2000). Sorghum is considered to have a remarkable genetic variability and is classified as Type I, II and III. Type I sorghums are generally white and do not possess a testa. These sorghums are preferred for most direct food uses but contain the lowest amount of phytochemicals. Type II sorghums, as defined in this paper but not necessarily elsewhere, contain a testa but do not have tannins. These sorghums may be called yellow, although most have a red colour appearance. Type III sorghums are also called brown, tannin or bird-resistant. They contain a pigmented testa and condensed tannins. Type II and III sorghums have the best nutraceutical potential because they exert a high antioxidant capacity imparted by phenolics, anthocyanins and tannins. The genetic sorghum background is the main responsible of affecting phenolic composition although the environment also affects.

This paper summarizes the phytochemical profile of different types of sorghum genotypes. Phenolics, flavonoids, anthocyanins, tannins, carotenoids and antioxidant capacities were assessed in order to detect the most promising genotypes. The *in vitro* inhibition of human mammary, colon and hepatic cancer cell growth and *in vivo* hypocholesterolemic effects of different sorghums were assessed.

MATERIALS AND METHODS

Sorghum Samples

The genealogy of the set of sorghum samples is in Table 1. The set of sorghums were planted at College Station, TX, in 2006. The samples also contained selected brans obtained after decortication using an IDRC mill equipped with abrasive disks. In the hypocholesterolemic study, two sorghum brans from black (Shawaya) and high tannin (Sumac) sorghums were used.

Table 1. Identification of sorghum samples.

Sample	Line designation	Type		Sample	Line designation	Type
1	ATX 635xRTX 436	Grain		14	Shawaya (Mostly Black)	Grain
2	SC748	Grain		15	Shawaya (Brown)	Grain
3	Hegari	Grain		16	NK 121A	Grain
4	TX2911	Grain		17	NK 180	Grain
5	TX430 Black	Grain		18	NK 8830	Grain
6	PI Black Tall	Grain		19	XM 217	Grain
7	Sumac	Grain		20	EBA 3	Grain
8	Sumac Bran	Bran		21	SC 575	Grain
9	TX430 Black Bran	Bran		22	SC 103	Grain
10	SC719-11E	Grain		23	SC 630 II	Grain
11	SC650	Grain		24	SC 1038	Grain
12	BRON 176	Grain		25	SC 630 II	Grain
13	SC109-14E	Grain				

Extraction

Samples of grain or brans were extracted with 80% methanol for 4 hr at room temperature under continuous agitation (200 rpm).

Determination of Phenolics, Anthocyanins, Flavonoids and Tannins.

The total phenols were determined following the Folin-Ciocalteu method of Singleton et al., (1999). Absorbance was measured at 750 nm using a UV/VIS spectrophotometer. The pH differential method of Fuleki and Francis (1968) was used to determine the content of crude anthocyanins. Absorbance was measured using a UV/VIS spectrophotometer at two wavelengths (514 and 700 nm). Flavonoids were measured using the modified colorimetric method of Govindarajan and Mathew (1965) and tannins were measured using the Vanillin HCl assay (Price et al., 1978).

Antioxidant Capacity

The antioxidant capacity was determined according to the method described by Prior et al., (2003). The peroxide radicals were produced by AAPH, using fluorescein as substrate and Trolox as standard. A Synergy HT microplate reader was used with fluorescence filters set to an excitation wavelength of 485 ± 20 nm and an emission wavelength of 530 ± 25 nm.

Cancer Cell Culture.

Three different cancer cell lines were used: mammary (MCF-7), colon (Caco2) and hepatic (HepG2). Cancer cells were maintained in DMEM-F12 medium containing 10% Foetal Bovine Serum and grown in an incubator set at 37°C, 80% relative humidity and 5% CO_2. The CellTiter 96® AQueous Cell Proliferation Assay was used to determine viability. Plates of 96-wells were prepared with 100 µl of a suspension containing 5 x 10^4 cells/ml of at least 12 h before adding the various sorts of extracts. Extracts were adjusted with cell growth medium to 2 mM and 100 µl of these solutions were added. After 24 h incubation, 20 µl of CellTiter were added and absorbance measured at 490 nm in a microplate reader. All extracts were tested at a concentration of 1mg/ml. Cell viability was calculated according to Mossman (1983).

Animal Trials

Four male and four female Gold Syrian hamsters *(Mesocricetus auratus)* weighing ~100 g were randomly assigned to 6 treatments and housed individually in metabolic cages. Hamsters were

kept in a 25°C room with a 12-h light:dark cycle and had free access to food and water throughout the duration of the 6-wk experiment. Hamsters were fed with a high fat and cholesterol diet. Two of the experimental diets were supplemented with 3.5% bran from either Sumac or Shawaya sorghums and the other two with the solids extracted with 80% methanol. At the end of week six, the food was removed 12 hr before sacrifice. Hamsters were anesthetized with chloroform, and blood taken via intracardiac puncture and then terminated by neck dislocation. Blood was obtained and placed in tubes containing 10 mg EDTA. Then, the liver and gall bladder were surgically removed. Red blood cells were removed by centrifugation at 3,500 rpm for 7 min al 4°C. Plasma (~2-4 mL) was recovered and analyzed for total cholesterol using a commercial enzyme kit assay (Randox Laboratories). Plasma HDL cholesterol concentration was measured after apolipopotein B precipitation, and non-HDL cholesterol (VLDL + LDL) was calculated by difference. Aliquots of frozen liver were minced and lipids extracted into chloroform:methanol (2:1, v:v) according to the method of Folch et al., (1957). Total cholesterol, and triglycerides were determined using commercial enzyme kit assays (Randox Laboratories). Gallbladder bile was collected using an insulin syringe and bile cholesterol and phospholipids determined using the modified method of Fiske and SubbaRow (1925).

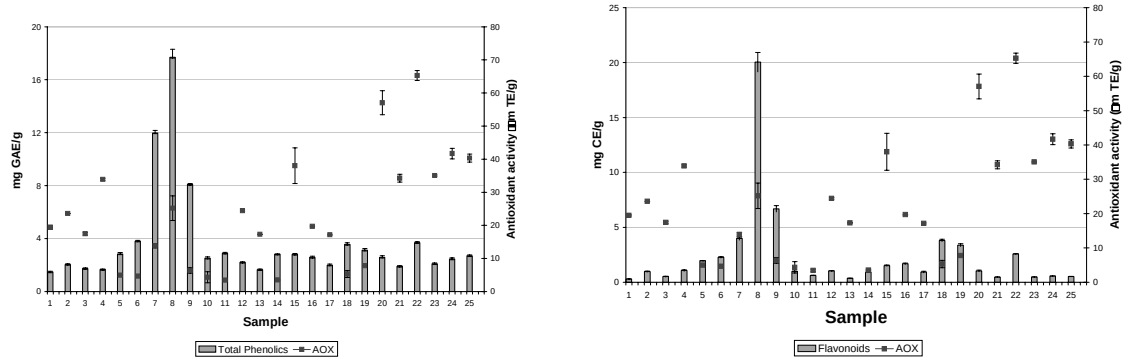
Statistical Analyses

Statistical analyses were performed on a personal computer using SigmaStat (SPSS Science, Chicago, IL). Treatment differences ($p < 0.05$) were determined using ANOVA.

RESULTS AND DISCUSSION

Phytochemical Profile and Cancer Inhibition of Different Sorghums

Figure 1 shows total phenols, flavonoids, anthocyanins and tannins of the 25 different types of sorghum grains or brans listed in Table 1. Entry 8 (Sumac bran from a Type III sorghum) was rich in total phenols, flavonoids, anthocyanins and tannins. Interestingly, this product had less antioxidant activity compared to entries 4, 20, 21, 22, 23, 24, and 25. Entries 6, 8 and 9 contained the highest amount of anthocyanins. Only entries, 7, 8, 15, 16, 18, 19 and 22 contained significant amounts of tannins. The different sort of phenolics contained in each type of product exerted different antiproliferative activities against mammary, colon and hepatic cancer cell lines (Fig. 2).



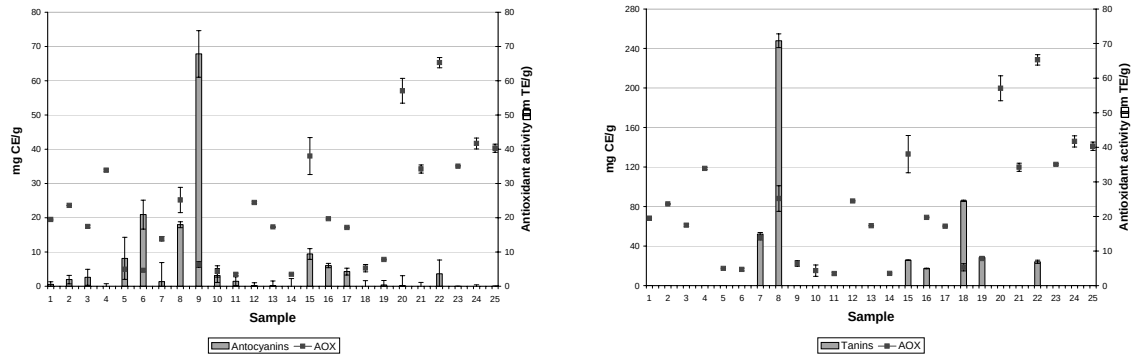


Figure 1. Total phenolics, flavonoids, anthocyanins, tannins and antioxidant activity of sorghum samples.

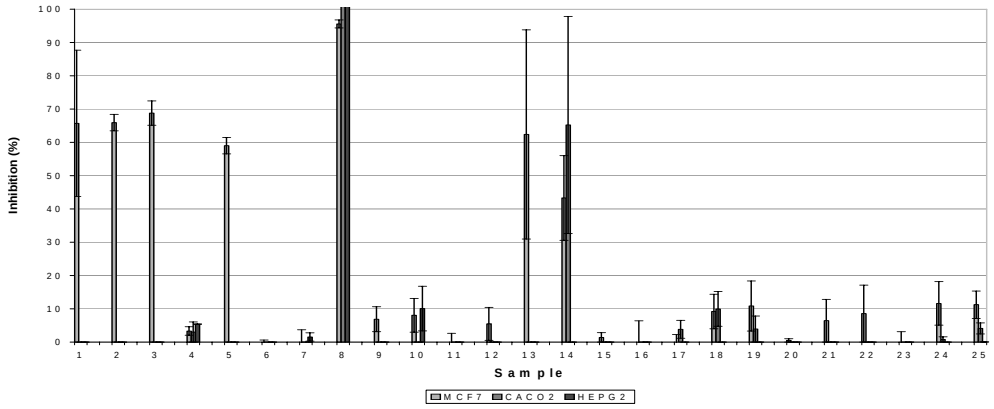


Figure 2. Effect of sorghum type on citotoxicity (expressed as percent inhibition) in different cancer cell lines: MCF-7 (breast cancer), Caco2 (colon cancer) and HepG2 (hepatic cancer).

Undoubtedly, the Sumac bran (entry 8) was the source that showed the best potential because inhibited more than 95% of mammary cancer cell growth and 60% colon and hepatic cancer cell growth. This product should be studied more extensively in order to find the most bioactive compounds.

Phytochemical Profile and Hypocholesterolemic Effects of Sumac and Shawaya Brans

The high tannin Sumac bran contained at least 5 times more total phenols compared to the black Shawaya bran (Table 2). However, the black Shawaya bran, although devoid of tannins, contained higher amounts of anthocyanins and antioxidant capacity. Results indicated that these two brans had higher antioxidant capacity and phenolic contents compared with the values reported by Awika et al. (2003) for the same varieties. Differences could be attributed to environmental effects or extent of decortication. Tannins and anthocyanins consistently impart higher antioxidant activity *in vitro* than other types of phenols (Hagerman et al., 1998, Dicko et al., 2006). Anthocyanins were the major extractable phenols from black Shawaya bran; hence, they were probably the major contributors to the high observed antioxidant activity or ORAC value (Table 2) (Awika and Rooney, 2004). The sorghum brans showed significantly higher values than fruits known to have high antioxidant capacity (Awika et al., 2003, Wu et al., 2004).

The high ORAC values observed in both sorghum brans demonstrate their high potential as a source of natural antioxidants to combat oxidative stress. These sorghum fractions can provide high antioxidant properties when used as ingredients in cereal-based foods. Plasma total cholesterol concentration was highest for the control animals and significantly lower in hamsters fed the Shawaya extract and high tannin Sumac bran (Table 3). Plasma LDL cholesterol concentrations were significantly increased in hamsters fed the Control diet. Interestingly, the best Non-HDL/HDL ratio was observed in animals fed the high tannins Sumac bran and the corresponding extract. Hamsters fed all the experimental diets contained lower amounts of bile cholesterol and phospholipids, indicating that both sorghums had a positive effect on hepatic metabolism.

CONCLUSIONS

Both high tannin and black sorghum brans and their respective extracts had hipocholesterolemic properties and therefore potential to combat cardiovascular disease. The advantage of sorghum is that is widely grown and can be produced massively in many countries around the world, it is easy to decorticate so to obtain the pericarp and testa rich in phytochemicals. Sorghums should receive more attention as a source of health-promoting phytochemicals.

Table 2. Phenolic composition and antioxidant capacity of two sorghum brans used in the hypocholesterolemic study

Bran	Total Phenols(mg gallic acid Equiv./g)	Flavonoids (mg catechin equiv./g)	Anthocyanins (mg catechin equiv./g)	Tannins (mg catechin equiv./g)	ORAC [a] (mmol Trolox equiv. /g)
High Tannin Sumac	17.69±0.32	5.77±0.08	9.29±7.2	68.45±1.76	25.19±1.13
Black Shawaya	2.81±0.02	3.24±0.17	13.16±7.75	0.00±0.00	38.02±0.31

[a] Oxygen radical absorbance capacity, fluorescein used as probe.

Table 3. Plasma and bile lipid concentration in hamsters fed for 6 weeks two different sorghum brans and their 80% methanol extracts[1]

Treatments	Plasma Cholesterol			Bile	
	HDL mmol/L	non-HDL mmol/L	Non-HDL/HDL	Cholesterol mmol/L	Phospholipids mmol/L
Control	2.88 ± 0.22	2.54 ± 0.16	1.00 ± 0.1	8.42 ± 1.6	1.60 ± 0.1
High Tannin Sumac Bran	2.76 ± 0.28	2.25 ± 0.34	0.76 ± 0.15	5.29 ± 1.7	0.97 ± 0.2
Black Shawaya Bran	2.26 ± 0.17	2.29 ± 0.31	1.38 ± 0.34	5.75 ± 1.6	0.90 ± 0.1
High Tannin Sumac Extract	2.84 ± 0.09	2.27 ± 0.48	0.87 ± 0.19	5.16 ± 1.9	1.03 ± 0.2
Black Shawaya Extract	2.23 ± 0.19	2.60 ± 0.54	1.09 ± 0.29	3.85 ± 0.7	0.95 ± 0.1

[1] Values are means ± SE.

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