CHEMICAL COMPOSITION AND ANTIOXIDANT EFFECTS OF EXTRACTS FROM SORGHUM FLOUR AND BRAN

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INTRODUCTION

Sorghum [*Sorghum bicolor* (L.) Moench] is an important food crop grown on a subsistence level by farmers in the semi-arid tropics of Africa and Asia. It is therefore important for food security in these regions (ICRISAT/FAO, 1996). The potential health benefits of sorghum are increasing in focus due to the presence of phenolic antioxidants in the grain (Awika and Rooney, 2004). These phenolic antioxidants may be classified into three main groups, namely phenolic acids, flavonoid-type compounds and condensed tannins (Waniska, 2000). Though all sorghum types contain phenolic acids and flavonoids, it is only varieties with a pigmented testa that contain condensed tannins. Most of the research done to date on characterization of phenolic antioxidants in sorghum has relied extensively on extraction of these compounds with organic solvents. However, this may not be of relevance if possible antioxidant effects of such extracts in cells from human tissue organs are to be investigated. For such studies, the use aqueous extracts or enzyme hydrolysates from the grain may be more appropriate. The objectives of this study were to determine the chemical composition of water extracts and extracts obtained by simulating gastric conditions from flour and bran samples of sorghum, and to determine the relation to their *in vitro* antioxidant effects.

MATERIALS AND METHODS

Preparation of Extracts

Sorghum flours of 90% and 60% extraction rate and their brans were prepared from a condensed tannin sorghum variety (PAN 3860). Water extracts and extracts obtained by simulating gastric conditions were prepared as described by Baublis et al. (2000) and Liyana-Pathirana and Shahidi (2005). An acidified methanol (1% HCl in methanol) extract of the bran flour was also prepared. All extracts were freeze-dried before analyses.

Determination of In Vitro Antioxidant Activity

In vitro antioxidant activities of the extracts were determined using the Trolox equivalent antioxidant capacity assay ($ABTS^{\bullet+}$ radical scavenging) as described by Awika et al. (2003).

Fourier Transform Infrared (FTIR) Spectroscopy

FTIR spectra of the extracts were collected in the dry state using a Bio-Rad FTS Spectrometer (Bio-Rad, UK).

UV-Visible Spectroscopy

Extracts were re-dissolved in either water or methanol to a concentration of 1 mg/ml. Acidified and alkaline variants of the water and methanol solutions of the extracts were also prepared. A Hewlett Packard HP 8452A diode array spectrophotometer (Hewlett Packard, UK) was used to collect UV-Visible spectra of the solutions in the wavelength range of 190 – 830 nm.

RESULTS AND DISCUSSION

Table 1. Trolox equivalent antioxidant capacity (TEAC) of water and gastric-simulated extracts of PAN 3860 sorghum bran and flour

Sample	TEAC (μmol/g)
Water extracts	
PAN 3860 bran (10% of whole grain)	1077 a (3)
PAN 3860 bran (40% of whole grain)	420 b (8)
PAN 3860 flour (90% extraction)	754 c (14)
PAN 3860 flour (60% extraction)	320 d (1)
Gastric-simulated extracts	
PAN 3860 bran (10% of whole grain)	1361 e (1)
PAN 3860 bran (40% of whole grain)	465 f (12)

Water extracts from the flour samples (90% and 60% extraction rate) had lower antioxidant activity than their corresponding bran samples (Table 1). The 10% bran water extract had higher antioxidant activity than the 40% bran water extract due to the fact that the latter had a relatively higher proportion of endosperm components. The water extract from flour of 90% extraction rate had higher antioxidant activity than the extract from flour of 60% extraction rate due to the fact that the latter had a greater proportion of the bran removed. These results indicate that the compounds contributing to antioxidant activity are concentrated in the bran. The extracts prepared simulating gastric conditions had higher antioxidant activity than their corresponding water extracts. The acid and alkaline conditions during extraction could have released some bound antioxidant via hydrolysis leading to higher antioxidant activity. Similar results have been reported in wheat flour (Liyana-Pathirana and Shahidi, 2005) and breakfast cereals (Baublis et al., 2000).

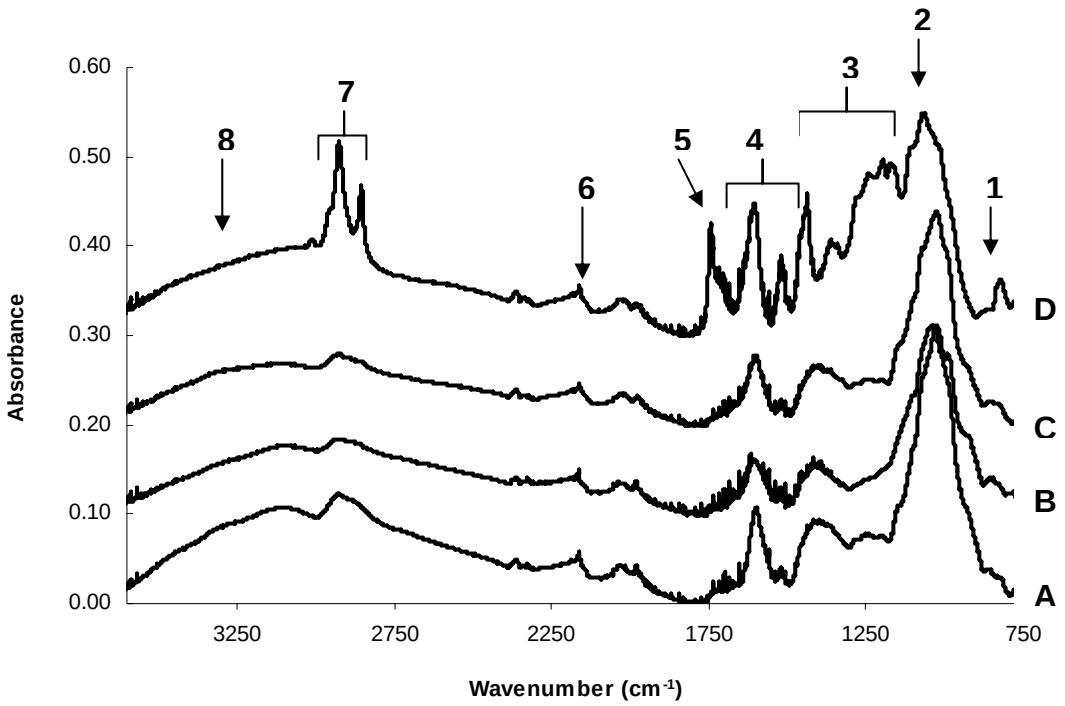

Figure 1. FTIR spectra of extracts from sorghum bran and flour.

A – Water extract from PAN 3860 bran (10% of whole grain); B – Water extract from PAN 3860 flour (90% extraction); C – Gastric-simulated extract from PAN 3860 bran (10% of whole grain); D – Acidified methanol extract from PAN 3860 bran (10% of whole grain).
Spectral assignments: 1 – C-H def (aromatics); 2 – C-O str (sugars); 3 – C-O str and O-H def (phenolics); 4 – C-O str (amides); 5 – C-O str (carbonyls); 6 – N-H str (amino acids); 7 – C-H str (aldehydes); 8 – O-H str (phenolics and sugars), C-H str (aromatics), N-H str (amines) (Kemp, 1987).

The FTIR spectrum of the acidified methanol extracts (Figure 1D) gave more intense peaks at frequencies assigned to aromatics (region 1), phenolics (region 3), amides (region 4), carbonyls (region 5), and aldehydes (region 7) compared to the spectra of the aqueous extracts (Figures 1A, B and C). This shows that the chemical composition of any extract is dependent on the solvent used for extraction. Figure 1 shows that acidified methanol extracts a wider range of compounds, mainly aromatic and phenolic compounds than water. It may therefore be hypothesised that the acidified methanol extract may have higher antioxidant activity than the aqueous extracts. There was no difference in the FTIR spectra of the water extracts (Figure 1A and B) compared to the gastric-simulated extract (Figure 1C). In this regard, the FTIR technique was not diagnostic enough to differentiate between these extracts in relation to their antioxidant activity. The absorptions due to sugars (region 2) and amides (region 4) were strong in the spectra of the aqueous extracts. This suggests that the aqueous extracts were composed of compounds including sugars, small peptides and possibly some low molecular weight phenolic compounds, most likely phenolic acids.

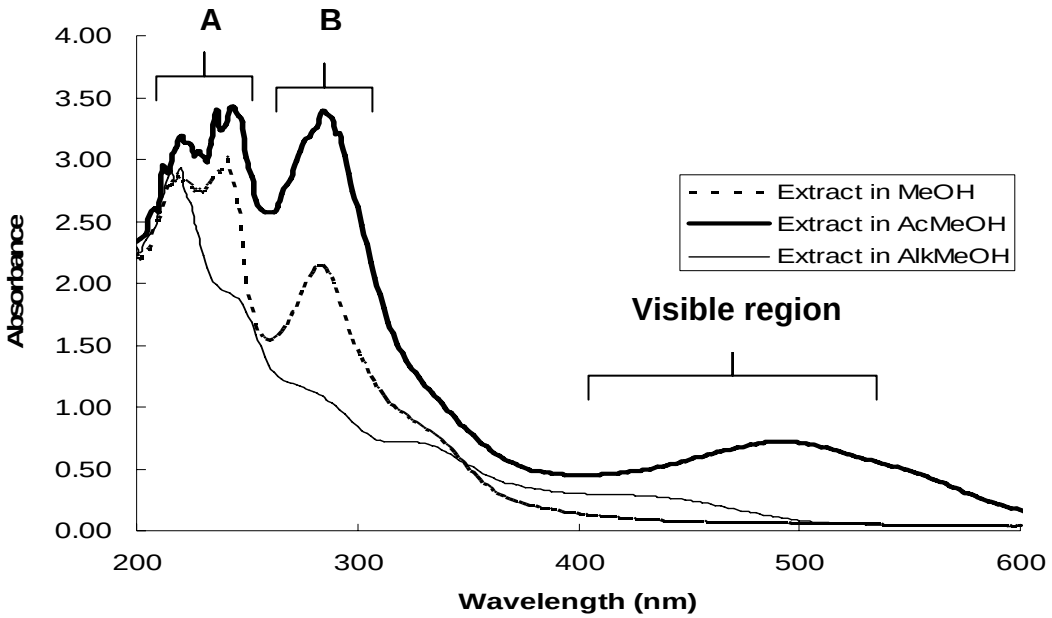

Figure 2. UV-Visible spectra of water extract of PAN 3860 sorghum bran (10%) in methanol (MeOH), acidified methanol (AcMeOH) and alkaline methanol (AlkMeOH).
Region A – 200-240 nm; Region B – 270-285 nm; Visible region – > 400 nm

The extracts were re-dissolved in methanol and acidified and alkaline variants were also prepared for UV-Visible spectroscopy. The spectra showed absorptions at 200 – 240 nm, 270 – 285 nm and in the visible region (Figures 2 and 3). Absorption in the 200 – 240 nm region may be attributed to various chromophores including C=C of various compounds, C=N (probably due to peptides and amino acid side chains), C=O of carbonyl compounds (which may include sugars and amino acids) and the benzene ring (probably from aromatic compounds) (Kemp, 1987). Absorption in the 270 – 285 nm region may be attributed to electronic transitions of benzene and its derivatives (which may include various aromatic compounds such as phenolics). The intensity of this band was higher for the bran extract (Figure 2) than for the flour extract (Figure 3) indicating that these components were higher in the bran. The bran extract showed a more intense absorption in the visible region compared to the flour extract (Figures 2 and 3). This suggests the presence of anthocyanin pigments (most likely 3-deoxyanthocyanins) in the bran extract (Awika et al., 2004).

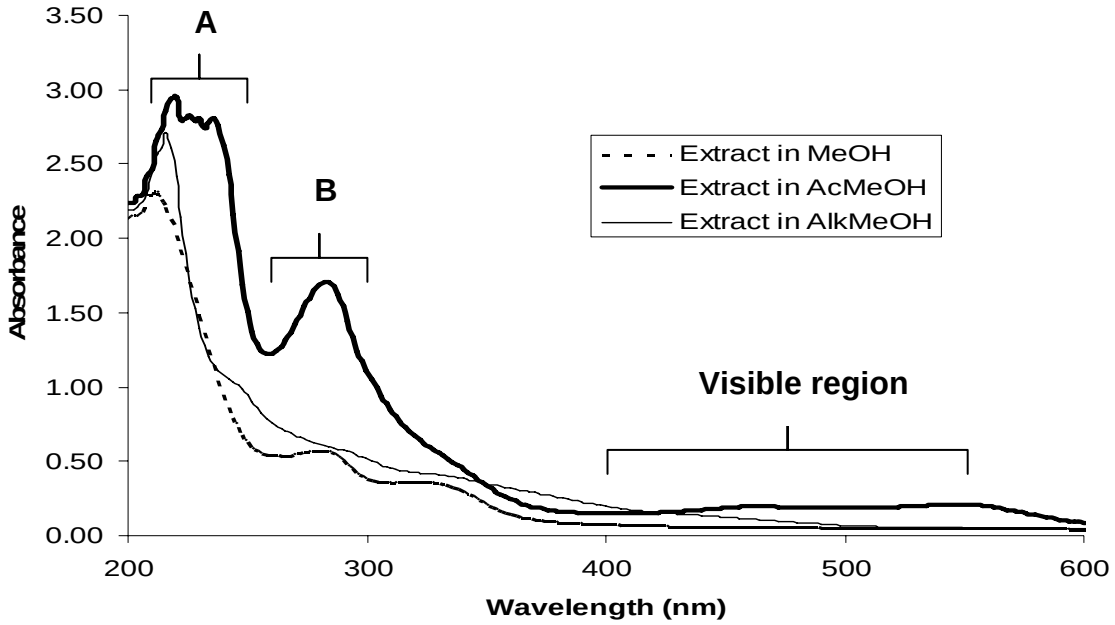

Figure 3. UV-Visible spectra of water extract of PAN 3860 sorghum flour (90% extraction) in methanol (MeOH), acidified methanol (AcMeOH) and alkaline methanol (AlkMeOH). Region A – 200-240 nm; Region B – 270-285 nm; Visible region – > 400 nm

These 3-deoxyanthocyanins may also have a significant influence on the observed high antioxidant activity of the sorghum bran extracts (Awika et al., 2004).

CONCLUSIONS

Water extracts from sorghum bran have higher antioxidant activity than water extracts from sorghum flour due to the fact that the compounds that contribute to antioxidant activity (phenolic compounds) are concentrated in the bran. Extracts prepared from the bran and flour by simulating gastric conditions, have higher antioxidant activity than their corresponding water extracts. Though water extracts from sorghum flour and bran may contain a wide variety of compounds including sugars, small peptides and some phenolic compounds, acidified methanol does extract a wider variety of compounds (especially aromatics) and in higher amounts than water. Water extract from sorghum bran contains pigments (possibly 3-deoxyanthocyanins) which may contribute significantly to the antioxidant activity of the bran.

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