ANTI-NUTRIENTS OR ANTI-OXIDANTS IN CEREAL GRAINS: AN EVALUATION OF THE COMPOSITION AND FUNCTIONALITY OF PHENOLIC COMPOUNDS WITH SPECIAL REFERENCE TO SORGHUM AND BARLEY

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Phenolic compounds (phenolics) in cereal grains encompass a diverse group of secondary plant metabolites. They can be conveniently divided into three broad groups, phenolic acids, flavanols, and polymeric flavanols including condensed tannins. Agronomically, the presence of phenolics is associated with diminished pre-harvest losses due to bird predation and post-harvest losses due to storage pests. However, tannins bind proteins, carbohydrates and minerals, thereby affecting the nutritional and functional value of the bound constituents. Phenolics may also impart undesirable colours in grain products during food processing. Recent investigations using sorghum grains have focused on examining the types and levels of phenolic compounds as constituents that adversely affect nutritional and sensory quality of food. The protocol included identification of widely cultivated sorghums, determination of phenolic compounds, and investigations on effective methods of processing tannin-rich sorghums. The primary objective is to improve the acceptance and utilization of sorghum for food as well as overall food security. The use of chemical treatments to reduce phenolics, reduce the enzyme inhibitory power of sorghum tannins and improve dry- and wet- milling properties of sorghum is discussed. Data is presented on phenolic compounds in barley. The functionality of cereal phenolics as anti-oxidants is also discussed.

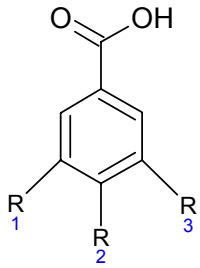
INTRODUCTION

Historically, Africa's indigenous cereal grains including sorghum have been a major food for humans and other animals and as constituents of nutritional and technological importance, cereal proteins and carbohydrates have been studied extensively. Among cereal grains, some sorghum and barley varieties contain high levels of phenolic compounds. Phenolic compounds (Figure 1) including phenolic acids, flavonoids, flavanols and proanthocyanidins (polymeric flavanols, also referred to as condensed tannins) are secondary plant metabolites naturally present in cereals and other plants as minor non-nutritive components[1]. Agronomically, the presence of phenolics is associated with diminished pre-harvest losses due to bird predation and post-harvest losses due to storage pests. However, phenolics bind proteins, carbohydrates and minerals, thereby affecting the nutritional and functional value of the bound constituents. Of major nutritional concern is the ability of tannins to bind strongly to large proteins and to proteins high in proline thereby reducing protein digestibility[2]. However, using both *in vitro* and *in vivo* approaches, Elkin et al[3] demonstrated that tannins are not the only grain components responsible for variations in the availability of nutrients in sorghum cultivars with similar tannin contents. Phenolics may also impart undesirable colours in grain products during food processing. Recent evidence indicates that consumption of foods rich in phenolics may help reduce the risk of strokes, coronary heart disease, certain cancers and liver disorders through their antioxidant activity[4]. It is most likely that, provided there is a sufficient bioavailability, phenolic compounds will play a major role in determining the antioxidant potential of cereal foods. The abundance of polyphenol-rich sorghums in Southern Africa[5] sparked our interest in investigating effective methods of processing the available varieties so as to improve the acceptance and utilization of sorghum for food as well as overall food security.

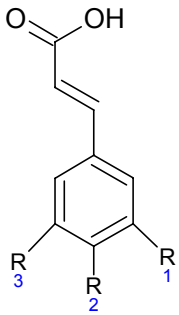
PHENOLIC COMPOUNDS IN CEREAL GRAINS

Cereal phenolics are primarily located in the grain outer layers. Phenolic acids in sorghum include benzoic and cinnamic acid derivatives[6]. Table I shows the total phenolic content of Zimbabwean sorghums determined using the vanillin assay[7] and the acid butanol assay[8] and high performance liquid chromatography[6]. Varieties (*DC-75*, *Mutode* and *Chirimaugute*) identified as containing high phenolic contents represent a fourth of the sorghums widely grown in Zimbabwe. Pericarp colour is not correlated to grain phenolic content; however, a significant positive correlation exists between grain phenolic content and pericarp thickness presumably due to the additional pigmented testa layer[5]. High-tannin sorghum varieties are preferred by some industrial sorghum maltsters (for example, in Zimbabwe, Botswana and South Africa) as the grain is more resistant to mould infection during the moist, warm conditions (95-100% RH, 25-30^{o}C) used for malting. Furthermore, tannin-containing sorghum varieties (*DC-75* and *Mutode*) accumulate higher levels of reducing sugars and free amino acids than tannin-free sorghum varieties (*SV2* and *Chihumani*)[9].

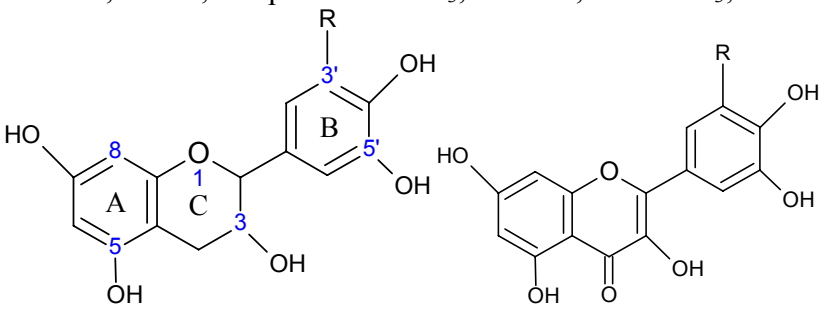
Barley contains 0.2 to 0.4% phenolics by weight of grain[10]. Phenolic acids in barley grain include benzoic acid (*p*-hydroxybenzoic acid, vanillic, and protocatechuic acids) and cinnamic acid derivatives (caffeic, coumaric, ferulic, and chlorogenic acids)[11]. Simple



Benzoic acid derivatives: protocatechuic: R1=H, R2=R3=OH; Gallic: R1=R2=R3=OH; *p*-hydroxybenzoic: R1=H, R2=OH, R3=H; vanillic: R1=H, R2=OH, R3= OCH_3.

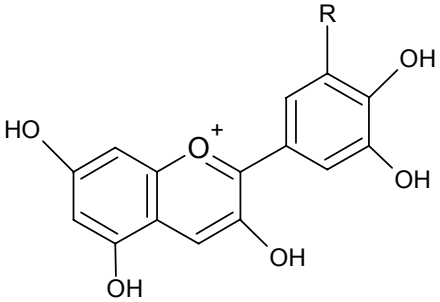


Cinnamic acid derivatives: *p*-coumaric: R1=H, R2=OH, R3=H; ferulic: R1= OCH_3, R2=OH, R3=H; sinapic: R1=OCH_3, R2=OH, R3=OCH_3; caffeic: R1=R2=OH, R3=H.



Flavonoids

Flavan-3-ols: catechin: R=H; gallocatechin: R=OH.
Proanthocyanidins of flavan-3-ols: linkage positions 4➔8 or 4➔6



Anthocyanins: cyanidin: R=H; delphinidin: R=OH.

Figure 1 Phenolic compounds in cereal grains

Variety[**]	Pericarp colour	Pericarp thickness, (m x 10^{-5})[¶]	Vanillin-HCl, (CE)[‡]	Butanol-HCl, (A/g)[‡]	Total Free Phenolic Acids, (μg/g)[†]
DC-75	red	6.6	6.29	45.88	636
Chirimaugute	white	5.3	3.78	29.31	563
Mutode	red	8.2	3.23	28.30	454
Mukadziusaenda	red	4.1	0.66	1.62	278
Chibonda	white	7.0	0.52	1.04	291
Iganu	red	3.0	0.36	1.21	491
Ntelwa	red	3.4	0.36	1.05	412
Tsveta	red	3.2	0.32	1.67	567
Katandanzara	white	2.4	0.19	0.34	117
Mukadziusaenda	white	2.8	0.18	0.42	257
Nyamidzi	white	5.2	0.12	0.87	223
Kasvikisire	white	3.7	0.05	0.25	206
Chihumani	white	2.3	0.03	0.19	137
SV2	white	3.4	nd	0.20	193
SV1	white	2.0	nd	0.20	210
NL330	white	3.9	nd	0.14	241
Mean		4.1	1.01	7.04	330

[**]Listed in order of decreasing catechin equivalents (CE) as measured by the vanillin assay.
[¶]Pericarp thickness
[‡]mg CE per 100 mg sample.
[‡]Absorbance units at 550 nm per g sample.
[†]Total free phenolic acids expressed as protocatechuic acid in μg per g sample.

Table I Kernel properties and phenolic composition of widely cultivated Zimbabwean sorghums (from Reference 5).

flavanoids (monomers, dimers and trimers) based on catechin and gallocatechin units account for 58-68% of the total phenolics[12].

Regarding functionality as health ingredients, the favorable redox potentials and the relative stability of their phenoxyl radical make phenolics good candidates as antioxidants[13]. Phenolic acids found in cereal grains are antioxidants *in vitro*[14] While many naturally occurring simple phenolics scavenge radicals as effectively as vitamins A and E when tested *in vitro*, more complex phenolics such as free- or protein-complexed proanthocyanidins seem to be most effective[15]. It is of some interest then that the proanthocyanidins found in barley exhibit antioxidant activity[16]. Recently it has been suggested that proanthocyanidin dimers and trimers could be absorbed *in vivo*[17]. However, there is still a paucity of studies on the bioavailability of phenolics.

Although the typical aggregate phenolic content of sorghum and barley is now known, the natural variations existing among the types of phenolics are poorly understood. There is also a dearth of studies on the chemical forms of phenolic compounds upon processing. The major limitations on all methods of analysis are the

different responses given by different phenolics and the difficulty of procuring an appropriate standard[18]. Fractionation of phenolic compounds using a combination of liquid chromatography with Sephadex LH-20 and RP-HPLC[19] is useful for studies on structure – function relationships among sorghum and barley phenolics. Given that the type of phenolic compound will influence biological properties[20], it is important to be able to fractionate cereal phenolics efficiently and to identify fractions with certain beneficial health effects.

EFFECT OF PROCESSING ON PHENOLIC COMPOUNDS

Attention is repeatedly drawn to the use of locally produced cereals in Southern Africa for food processing. However, the lack of ideal grain quality parameters[5] compounded with the scarcity of suitable processing technologies at household level should serve as an impetus for grain scientists to identify methods of utilizing the available cereals. During processing, phenolics other than those endogenous in cereal grains may be formed as by-products of enzymatic or thermal degradation or as products of polymerization of simple phenolics. Three Zimbabwean varieties representing tannin-free (*SV2*), medium-tannin (*Chirimaugute*) and high-tannin (*DC-75*) sorghum types have been used as model cereals to follow changes in phenolics when grains are treated with food grade chemicals prior to malting or milling. The use of water, HCl (0.25 M), formaldehyde (0.017 M) and NaOH (0.075 M) during steeping of tannin-containing varieties reduce phenolic content of the grain[21]. However, polyphenols remain inhibitory to the enzymes even after malting when water or HCl has been used for steeping. While malting alone does not effectively reduce the enzyme inhibitory power of the tannins, treatment with NaOH or formaldehyde is effective for purposes of increasing available diastatic power (DP) in sorghum malt from tannin-containing varieties[21]. Similarly, conditioning treatments using dilute alkali reduce assayable phenolics and undesirable enzyme inhibition of the milled products[22].

Coarse milling of hulled barley followed by sieving results in higher phenolic content in the coarsest fraction in comparison to other milled fractions[12]. Sorghum offal or bran-enriched fractions obtained by simple machine- dehulling or roller milling of model cereals contain higher levels of phenolics than the meal[22]. Primary processing can be used to produce phenolic-enriched materials for further investigations on functionality. Although the colour of sorghum meal is not correlated to phenolic content, results on sensory evaluation of thick porridges prepared from Southern African sorghums containing red and white indicate that processing effects chemical change in endogenous phenolics pericarps (Figure 2). Panelists consistently show strong preferences for porridges from white sorghums. *Macia* and SV2 good examples of white sorghums that contain white pericarps and non-pigmented kernels. According to Kambal and Bate-Smith[23], flavonoid compounds are responsible for the pericarp colour of sorghum grains. The red, lemon yellow or red pericarp colour is under genetic control[24]. Tannin-containing varieties, *Chirimaugute* and *DC-75* give pink coloured starches[25] due to adsorption and retention of tannins by the starch[26]. None of the selected treatments could prevent the pink colouration of starch prepared from tannin-containing model cereals[25]. During processing, phenolics form complexes with proteins and carbohydrates through hydrogen bonds and hydrophobic interactions[27]. In addition, proanthocyanidins form covalent bonds with proteins through oxidative polymerization. Our postulate, using model cereals is the formation

of oxidized phenolic products and/or higher molecular weight polymers which are less reactive during processing of sorghum.

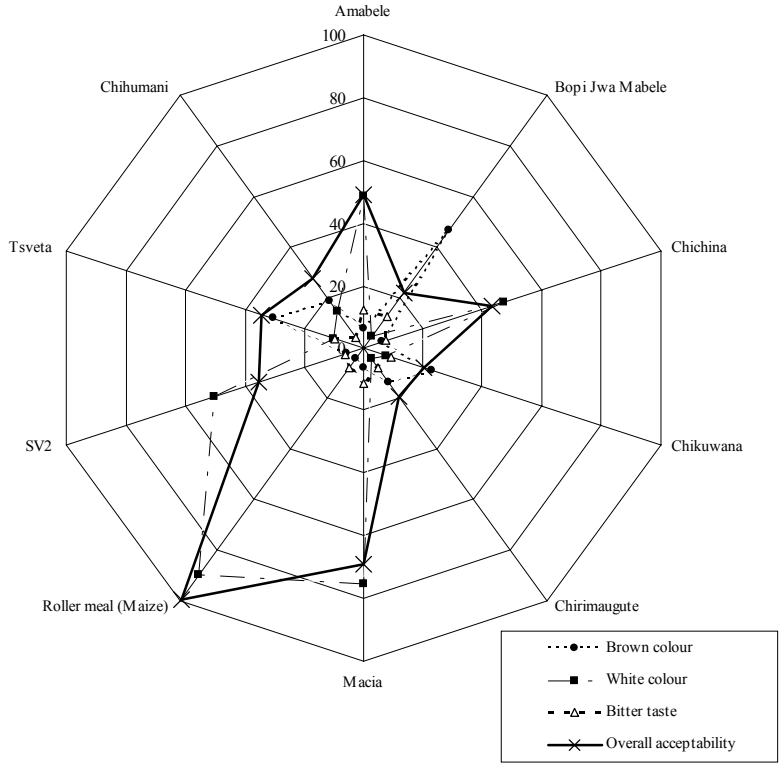


Figure 2 Spider chart of mean scores for maize (commerical roller meal, Zimbabwe) and sorghum sadza (thick porridges) from varieties containing white- (SV2, Chihumani, Macia, Chichina), red- (Tsveta, Chikuwana, Chirimaugute), coloured pericarps. Amabele (Zimbabwe) and Bopi Jwa Mabele (Botswana) are commercial sorghum meals (Beta et al 2001).

In studies using materials other than sorghum, cooking and baking results in the partial loss of phenolics[28]. Friedman[29] observed chemical changes in the absorption spectra of phenolics under the influence of pH. In food systems such as extruded products, phenolics are subjected to various degrees of heat-moisture treatments. Phenolics can become modified such that their solubility and functional group properties are altered. Phenolic-enriched grain and grain products are excellent candidates for investigations on functionality as antinutrients or antioxidants where the main objective is to optimize beneficial health effects of phenolic compounds.

CONCLUSION

The role of phenolic compounds in cereal grains as antinutrients or antioxidants is not yet definitive. Evidence has been presented showing the effect of processing on phenolics of model cereals. While such treatments are simple and effective, investigations on the chemical forms predominant in the products are needed. It is envisaged that the interaction of phenolics with other components during processing will affect their functionality as antinutrients or antioxidants. Characterization of the molecular structure - function relationships of cereal phenolics can lead to their development as natural antioxidants. However, there are scientific issues that need to be resolved pertaining to the characterization of phenolics and several steps are needed to validate their stability and value as natural antioxidants under food processing conditions that use heat and extremes of pH.

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