Sorghum Flavonoids: Unusual Compounds with Promising Implications for Health

Joseph M. Awika*

Soil & Crop Science Dept./Nutrition and Food Science Dept., 2474 TAMU, Texas A&M University, College Station, TX 77843-2474
*E-mail: jawika@ag.tamu.edu. Phone 979-845-2985.

Sorghum contains high levels of a diverse array of flavonoids, many of which are not typically found in other cereal grains. The high levels of compounds like the stable 3-deoxyanthocyanin pigments, proanthocyanidins, flavones, and flavanones in certain sorghum varieties is especially of interest both from a commercial and health perspective. Evidence suggests that the sorghum flavonoids produce specific health benefits that are not observed for other grains like corn, rice, or wheat. For example, epidemiological and laboratory evidence suggest superior chemoprotective properties of sorghum when compared to other grains. Other evidence indicates that sorghum components elicit anti-inflammatory response and other benefits not seen with other grains in vitro and in vivo. This chapter reviews the chemistry of the major flavonoids in sorghum, their unusual properties, and potential health benefits.

Part I: Occurrence and Chemistry

Introduction

Flavonoids are widely distributed secondary plant metabolites that have been extensively investigated for their health benefits. They are a class of phenolic compounds that share a basic C6–C3–C6 structure, consisting of two aromatic rings joined by a three carbon heterocyclic ring (Figure 1). More than 5000 flavonoids occur in nature differing in substitution patterns on the benzene rings as well as the heterocyclic ring. In general, flavonoids are grouped based on the heterocyclic ring structure; common classes include anthocyanins, flavanols,
flavones, isoﬂavones, ﬂavanones, ﬂavonols, and the polymeric proanthocyanidins. Flavonoids play an integral role in plant growth, reproduction and function. For example, most flower pigmentation in plants (believed to help attract pollinators) is derived from anthocyanins, whereas the high concentration of flavonoids in skin of most fruits, nuts and grains, help protect against pathogens, UV damage, and pests. Flavonoids are also produced inducibly by plants during stress, e.g., pathogen attack, herbivore-induced damage, etc, ostensibly as a natural defense mechanism (1).

![Flavan structure](image)

**Figure 1. Flavan structure, the backbone of flavonoid compounds; the B-ring attaches at position 3 for isoﬂavonoids and position 4 for neoflavonoids.**

In human nutrition, flavonoids have been long recognized for the important role they play in contributing to health benefits associated with fruit and vegetable consumption. Among the earliest documented health benefits of flavonoids was capillary wall strengthening effect reported by Rusznak & Szent-Gyorgyi (2). The observations led to the term ‘vitamin P’ being originally used to describe these compounds, probably due to a belief they played the same critical roles as vitamins in human. Besides fruits and vegetables, these compounds are found in relatively large quantities in many food products regularly consumed, e.g., red wine, fruit juices, tea, chocolate, among others. Though research on potential health benefits of flavonoids has been on-going for decades, widespread studies began in the 1990s, when direct evidence for their powerful antioxidant properties (as possible free radical scavengers in the body) became obvious. Consequently, part of health benefits that had been largely attributed to the antioxidant vitamins A and E in fruits and vegetables, or even alcohol (in the case of wine) could be more directly attributed to the phenolic compounds (3).

Even with the frenzy of research that began in the 1990s aimed at uncovering the health benefits of flavonoids and other phenolics related to their antioxidant properties in fruits, vegetables and related commodities, few studies focused on investigating antioxidant and health promoting properties of cereal flavonoids. This is partly because cereal grains in general contain low levels of phenolic compounds, especially flavonoids, and most the compounds are discarded with the bran fractions during milling operations. Besides, the health benefits of whole grain consumption were long believed to be largely due to the dietary fiber in bran. Most investigations of cereal grain flavonoids prior to the late 1990s were concerned with their relationship to plant and seed development and resistance
to various stresses, as well as impact on food product color and other sensory properties. Evidence that phenolic compounds may contribute significantly to the health benefits of whole grain consumption emerged when purified cereal bran fiber components, like cellulose, failed to produce similar effects seen for whole grain.

In sorghum, like most cereals, the phenolic compounds are mostly concentrated in the bran. However, sorghum generally contains much higher levels of flavonoid compounds than most other cereals or even fruits and vegetables (4). The type and level of flavonoid compounds in sorghum vary significantly by variety, and are controlled by a set of well documented genes (5). Particularly interesting is the fact that the yellow seed1 (ys1) gene found in most sorghum varieties controls the biosynthetic pathway that leads to accumulation of 3-deoxyflavanoid compounds (6). These compounds are not usually found in other cereal grains in meaningful quantities, and are partly responsible for the high antioxidant and other beneficial properties of sorghum relative to other grains (7, 8). The unusual flavonoid composition of sorghum and their high levels of accumulation in sorghum bran certainly make sorghum an interesting grain for healthy dietary applications or as a source of bioactive compounds. The chemistry of these compounds and some of their interesting properties are reviewed in the following sections.

3-Deoxyanthocyanins

Of all the sorghum flavonoids, perhaps the most uniquely interesting/intriguing are the 3-deoxyanthocyanins (Figure 2a). These compounds are responsible for most of the red to black pigmentation on sorghum grain, glumes, sheath, stem, and leaves. Their stability and other attributes have resulted in a growing interest by the food industry to use them as natural food colors. The 3-deoxyanthocyanins are analogous to the anthocyanins (Figure 2b) responsible for the blue, purple and red pigmentation on most fruits, flowers and vegetables. However, unlike the anthocyanins, the 3-deoxyanthocyanins are not substitution at the C-3 position. The 3-deoxyanthocyanins have a very limited distribution in nature, and are typically not present in most other cereal grains or food plants in meaningful quantities.

The synthesis of the 3-deoxyanthocyanin pigments in sorghum is controlled by a set of two genes, R and Y. A homozygous recessive yy will produce a white pericarp essentially devoid of any pigments, whereas a recessive rr gene will produce a yellow pericarp with very little 3-deoxyanthocyanins. Dominance at R and Y will result in a red pericarp (5); these sorghums accumulate significant levels of the pigments. Other interesting traits have also been observed in sorghum pigmentation; for example, some red sorghum varieties will turn black during grain maturation due to exposure to UV light, with a concomitant many-fold increase in 3-deoxyanthocyanin accumulation (9). The genetic basis for this response is not fully understood, but it suggests these sorghum varieties may be producing these compounds as a means of protecting the seed against UV radiation. Sorghum happens to be one of the few species of monocotyledons capable of synthesizing pigmented phytoalexins (secondary
metabolites produced as a result of mold invasion or other stresses); which are usually the 3-deoxyanthocyanins (10). Important differences between the anthocyanins and the 3-deoxyanthocyanins are worth discussing in some detail.

![Figure 2. Common 3-deoxyanthocyanin compounds found in sorghum (a) and their analog anthocyanins (b). 3-Deoxyanthocyanins (a): Apigeninidin based compounds, \( R_1 = R_2 = H \); luteolinidin based compounds, \( R_1 = OH, R_2 = H \); 7-O-methyl derivatives, \( R_3 = H/Glucose, R_4 = CH_3 \); 5-O-glucosides, \( R_3 = Glucose, R_4 = H \); Tricetinidin, \( R_1 = R_2 = OH \). Anthocyanins (b): Aglycons, \( R_3 = H \); glycosides, \( R_3 = sugar or acyl sugar (C-5 is also sometimes sugar substituted) \); Pelargonidin, \( R_1 = R_2 = H \); cyanidin, \( R_1 = OH, R_2 = H \); delphinidin, \( R_1 = R_2 = OH \).](image)

**Chemical Structure and Occurrence in Nature**

As previously noted, the most fundamental difference in structure of the 3-deoxyanthocyanins relative to the anthocyanins is the unsubstituted C-3 position (Figure 2a & b). Most anthocyanins in nature are glycosyl substituted (usually with one or more sugar molecules, with or without phenolic acids esters, among other possibilities) at the C-3 position. The glycosyl substitution at C-3 significantly contributes to improved stability of the anthocyanins since their aglycons (with –OH at C-3 position) are highly unstable. By contrast, 3-deoxyanthocyanins tend to naturally exist mostly as their glycons (3-deoxyanthocyanidins); with lower levels of O-glycysides and acyl-glycoside complexes at position 5 or 7. This may partly be due to the fact that these compounds are rendered relatively stable by the lack of a –OH group at the highly reactive C-3 position, and thus do not need to be glycosylated for stability. The chemical basis for the stability of the 3-deoxyanthocyanins is discussed in the following sub-section.

In sorghum, luteolinidin and apigeninidin (Figure 2a) are the primary aglycons found. These molecules are analogous to the cyanidin and pelargonidin anthocyanidin molecules, respectively (Figure 2b). The structural analogy has allowed for accurate documentation of the effect of 3–OR substitution on the chemical and biochemical properties of anthocyanin compounds over the years. Apart from luteolinidin and apigeninidin, the other major 3-deoxyanthocyanidin
of dietary importance is the tricetinidin (Figure 2a), a red pigment analogous to delphinidin, commonly found in black tea as an oxidative degradation byproduct of epigallocatechin gallate; this compound has not been identified in sorghum. In sorghum, the two 3-deoxyanthocyanin aglycons, apigeninidin and luteolinidin, also exist commonly as their 7-O-methyl derivatives (11, 12), even though 5-O-methyl forms have been reported (13). These O-methylated compounds generally have very similar chemical properties (e.g., stability and color absorption characteristics) to their base molecules, but somewhat different bioactive properties that may be relevant to sorghum variety selection for health applications (12) (see Section II). It is important to note that for widely distributed anthocyanins found in grains and other plants that are O-methylated, the substitution is typically on the B-ring, as is the case for 3′,5′-O-dimethyl substituted malvidin and the 5′-O-methyl substituted petunidin. However, uncommon anthocyanidins, including hirsutidin, and rosinidin (found in Catharanthus roseus) with O-methyl substitution on the A-ring have been found in some plants.

A few glycosides and acyl-glycosides have been identified in sorghum, even though many of these compounds are yet to be structurally identified judging by the number of unknown peaks on HPLC chromatograms we have observed for various sorghum pigment extracts. Presence of 5-O-glucosides of apigeninidin and luteolinidin were originally reported by Stafford (14). The authors also suggested presence of acyl-glycosides of the 3-deoxyanthocyanidins in sorghum; this was later confirmed by Hipskind et al (15) who identified a ferulic acid ester of arabinosyl-5-O-apigeninidin in sorghum. Polymeric 3-deoxyanthocyanins have also been reported in sorghum, though the precise structures are unknown. More recently, Khalil et al (16) isolated a symmetrical pyrano-3-deoxyanthocyanidin pigment (pyrano-apigeninidin-4-vinylphenol) from a red sorghum (Figure 3). Obviously the growing interest in the sorghum pigments for food and health applications will lead to increased research into unraveling new details about the composition of these compounds.

![Figure 3. A Pyrano-apigeninidin-4-vinylphenol recently identified in sorghum (16).](image-url)
In sorghum, the levels as well as relative proportions of apigeninidin and luteolinidin-based compounds is influenced by variety.genetics. For example, red pericarp sorghum grains with tan secondary plant color have much higher proportion of apigeninidin-based compounds, with only trace amounts of luteolinidin-based compounds whereas sorghums with rep/purple secondary plant color have a more balanced distribution of luteolinidin and apigeninidin-based compounds (9, 17). Specialty pigmented sorghums generally contain higher levels of pigments than other pigmented cereals (Table I), which points to an economic potential of sorghum as a source of natural food colorants.

Table I. (3-Deoxy)Anthocyanin content of pigmented sorghum relative to other cereal grains

<table>
<thead>
<tr>
<th>Commodity</th>
<th>Amount (µg/g)</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Black sorghum</td>
<td>1,000 – 2,800</td>
<td>(18)</td>
</tr>
<tr>
<td>Red sorghum</td>
<td>14-680</td>
<td>(9, 17, 18)</td>
</tr>
<tr>
<td>Lemon yellow sorghum</td>
<td>8-108</td>
<td>(19)</td>
</tr>
<tr>
<td>Black sorghum bran</td>
<td>4,700 - 16,000</td>
<td>(18, 20)</td>
</tr>
<tr>
<td>Blue barley</td>
<td>4</td>
<td>(21)</td>
</tr>
<tr>
<td>Red/purple maize</td>
<td>225 - 965</td>
<td>(21)</td>
</tr>
<tr>
<td>Blue/purple wheat grain</td>
<td>35-507</td>
<td>(21, 22)</td>
</tr>
<tr>
<td>Blue/purple wheat bran</td>
<td>108-485</td>
<td>(23)</td>
</tr>
<tr>
<td>Black rice</td>
<td>158-299</td>
<td>(24)</td>
</tr>
</tbody>
</table>

Accumulation of 3-deoxyanthocyanin pigments in sorghum grain is almost exclusive to the pericarp, as is the case for other cereal grains; this implies the sorghum bran which is in most cases a byproduct of grain processing could significantly increase in value as a concentrated source of these compounds. However when physiologically stressed, e.g., via fungal attack during grain maturation, the sorghum may synthesize a high concentration of the phytoalexin 3-deoxyanthocyanins at the site of attack, some of which may leach into the endosperm causing a blotchy appearance. This is a type of grain weathering, and is usually more apparent in sorghum cultivars with red or purple secondary plant color. Recent evidence indicates that purple plant sorghum sheath has an order of magnitude higher level of the 3-deoxyanthocyanins than found in bran (25). In fact these sorghum sheaths are widely used in West Africa as a source of commercial cosmetic dyes. Interest in using them for food applications is currently strong.
An important difference between the 3-deoxyanthocyanins and anthocyanins is the color properties. The 3-deoxyanthocyanins absorb visible light maximally at lower wavelength (typically below 500 nm at pH below 7.0) than the anthocyanins (usually above 510 nm). Thus the 3-deoxyanthocyanins appear yellow – orange whereas the anthocyanins appear red – blue. The two groups of compounds are consequently not necessarily substitutable, but would likely complement each other in food applications. However, an especially important distinction of the 3-deoxyanthocyanins is their relative resistance to a drop in molar absorptivity (color fading) as the pH increases when compared to anthocyanins. Both anthocyanidins and 3-deoxyanthocyanidins exist in the form of the orange-red flavylum cation (AH⁺), red-blue quinoidal base, and the colorless carbinol pseudobase and chalcone species (Figure 4). The relative concentrations of these species and the final color of the solution depend on the pH.

The predominant form of both anthocyanidins and 3-deoxyanthocyanidins at pH ≤1.0 is the AH⁺; this is also the form that has the highest molar absorptivity (produces most intense color). The AH⁺ becomes less stable as pH increases and transforms into the various forms via hydration (into colorless carbinol and chalcones) or deprotonation (into quinoidal forms). The degree of transformation depends on the solution pH, ionic properties, as well as the structure of the (3-deoxy)anthocyanin molecule. Thus the maximum color intensity and stability of (3-deoxy)anthocyanin compounds is achieved in strongly acidic environments where the AH⁺ is most stable.

As the pH increases, the color intensity of (3-deoxy)anthocyanin pigments drops significantly. For example, cyanidin lost 84%, while pelargonidin lost 95% of its absorbance when the pH changes from 1.0 to 3.0 (17, 18) (Table II). In fact the monomeric non-acylated anthocyanins lose almost all their absorbance at pH 4 – 5, due to near complete transformation of the flavylum cation to the colorless chalcones and carbinol pseudobases. On the other hand, the predominant sorghum 3-deoxyanthocyanidins, luteolinidin and apigeninidin were reported to lose only 20% and 38% of molar absorptivity as pH changes from 1.0 – 3.0, and retained significant absorptivity at pH above 4.0 (17, 18). Additionally, the 3-deoxyanthocyanins are relatively resistant to color degradation over time in presence of anthocyanin bleaching agents like ascorbate or sulfites (26, 27). Co-pigmentation, particularly with ferulic acid and tannic acid were found to markedly improve their stability further (28).
Figure 4. Transformation reactions of luteolinidin in aqueous solution at pH above 2.0. Due to the higher deprotonation constant ($K_a$) relative to hydration constant ($K_h$) of 3-deoxyanthocyanidins, the predominant reaction as pH increases is the deprotonation to colored quinoidal bases which help enhance color stability in mildly acidic solutions. Anthocyanins on the other hand, hydrate much more readily losing most color at pH 3 – 5.

The better stability of the 3-deoxyanthocyanins to pH change and hydrophilic attack compared to the anthocyanins has been attributed to the relatively hydrophobic nature of the heterocyclic ring of the 3-deoxyanthocyanins. For one, these compounds have lower hydration rate constants than ionization rate constants (29, 30); this implies that in aqueous solution, they more readily deprotonate as pH increases into the quinoidal bases which are themselves colored compounds. On the other hand, anthocyanins, which have on average three orders of magnitude higher hydration rate constants than the 3-deoxyanthocyanidins (29) are more prone to hydrophilic attack and thus transform mostly into the colorless carbinol bases as pH increases. In addition, the AH$^+$ of the anthocyanins loses stability at lower pH than the 3-deoxyanthocyanins. For example, in an experiment by Mazza and Brouillard (29) cyanidin-3,5-diglucoside hydrated readily at pH above 0.5 and existed as AH$^+$ and carbinol forms at 50% and 35% (pH 2.0), and 15% and 80% (pH 3.0), respectively, in equilibrium. The quinoidal forms did not reach 10% even at pH 6.0 in this experiment. On the other hand, equilibrium distribution of 3-deoxyanthocyanidins were markedly different under...
similar conditions; for example, apigeninidin existed almost exclusively as AH+ at pH 2.0, and as 85% AH+ and 10% quinoidal bases at pH 3.0 (29, 30). Thus it seems the relative hydrophobic nature of the 3-deoxyanthocyanidins results in a poor hydration of the pyrylium (heterocyclic) ring and contributes to their protection in aqueous system at lower pH.

Table II. Effect of increase in pH on relative molar absorptivity of 3-deoxyanthocyanidins and their anthocyanidin analogs. Adapted from Awika et al (17)

<table>
<thead>
<tr>
<th>pH</th>
<th>3-deoxyanthocyanidins</th>
<th>Anthocyanidins</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Luteolinidin</td>
<td>Apigeninidin</td>
</tr>
<tr>
<td>1.0</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>2.0</td>
<td>97</td>
<td>96</td>
</tr>
<tr>
<td>3.0</td>
<td>80</td>
<td>62</td>
</tr>
<tr>
<td>5.0</td>
<td>36</td>
<td>41</td>
</tr>
</tbody>
</table>

Values shown are percent absorbance normalized to absorbance in pH 1.0 buffer. Molar absorptivity based on absorbance at λ_max for each sample. Values of λ_max (nm) in pH 1.0 buffer: Luteolinidin, 482; apigeninidin, 468; cyanidin, 516; pelargonidin, 504.

Opportunities and Challenges of Using Sorghum Pigments as Natural Colorants

Even though sorghum pigments are relatively stable when compared to commercially available ones from fruits and vegetable (e.g., red cabbage) (27), there are practical hurdles that need to be addressed for these pigments to become commercially more feasible. For one, the compounds are still susceptible to bleaching at higher pH by ascorbic acid or bisulftes (both relatively common food additives), though to a lesser extent than the anthocyanins. However, new discoveries are being made on the pigment reactions and natural conformations that will significantly enhance their stability in presence of food additives. For example, in presence of pyruvic acid (and possibly acetic acid), the sorghum 3-deoxyanthocyanins form pyrano- structures that are very resistant to, for example, ascorbic acid bleaching. This is because the cyclic condensation reaction between C-4 and the -OH at C-5 blocks the C-4 position that is most susceptible to nucleophilic attack by ascorbate/sulfites. This same mechanism is believed to contribute to stability of wine pigments. The recent discovery of natural pyrano- forms of 3-deoxyanthocyanins in a red sorghum variety (Figure 3) is very interesting in this regard.

Another problem with sorghum grain pigments is their extractability. The pigments in bran are generally very difficult to extract in aqueous solvent under atmospheric conditions. In fact for most research reporting, sorghum pigments are often extracted in acidified methanol, which will extract at least 90% more
pigments than acidified water under similar conditions (31). Even then, most of the pigments remain in the bran, judging by the dark color left on residue even after soaking for 24 hr in acidified methanol. This implies that the reported values for pigment content of sorghum bran are highly underestimated. Improving pigment yield from sorghum bran in water often requires temperature and/or pressure manipulation. With growing commercial interest, newer more efficient extraction methods will undoubtedly be devised, e.g., use of cellulosic enzymes to break down the bran and easily release the pigments may become more feasible by borrowing from the rapid advances in biofuel-based cellulosic digestion technology. On the other hand, pigment extraction from non grain sorghum tissue, such as glumes or sheath, is much easier and can be readily achieved in water at room temperature. Given that glumes and sheath from purple plant sorghum contain especially high levels of the 3-deoxyanthocyanins (32, 33), they are a logical target for easy concentration of these compounds for commercial applications.

A third practical problem is related to the stability of these compounds; as previously explained, the 3-deoxyanthocyanins resist degradation in large part due to their resistance to hydration. This unfortunately leads to a higher tendency of these molecules to self associate and precipitate out of aqueous solution over time, particularly as pH rises to above 4.0 when the quinoidal forms become dominant. This may make the 3-deoxyanthocyanins less appealing for some applications. However, various mechanisms, some that are already widely used in the industry, are available to address this problem.

In all, the sorghum pigments are likely to contribute significantly to the natural food colorant market, especially as companies work to transition from the petroleum based synthetic dyes to the natural food colors, necessitated partly by consumer demands and more importantly by government regulation (especially in the European Union). Within the past 5 years or so, the use of natural colorants has sky rocketed, and in 2010 accounted for about one third of the more than $1.5 billion food colorant market. However, given natural colorants cost 10 – 20 times more than their artificial counterparts, the room for growth is enormous. Sorghum has added benefits in terms of higher concentration of pigments (Table I), better stability to storage, and ability to derive pigments from parts of grain/plant that are typically low value feed/waste. The fact that the sorghum pigments provide hue properties that are complementary to the anthocyanins and other natural colorants increases their importance.

**Flavones**

Flavones are a group of flavonoids that contain a 2-phenyl-1-benzopyran-4-one skeleton (Figure 5a). Flavones are mainly reported in herbs such as parsley and celery (34). However, sorghum accumulates flavones and their derivatives at nutritionally significant levels (Table III). In sorghum the two major flavones present are apigenin and luteolin, along with their 7-O-methylated derivatives (9, 14), and some glycosides. The fact that sorghum flavones are mostly apigenin and luteolin derivatives points to a possible common biosynthetic pathway of these compounds and the 3-deoxyanthocyanins. Accumulation of flavones is sorghum is
highly influenced by genetics; pigmented sorghum grain with tan secondary plant colors have much higher levels of these compounds than pigmented sorghums with red/purple secondary plant color. For example, tan plant sorghums with a pigmented pericarp had 60 – 386 µg/g flavones, whereas purple plant varieties with a pigmented pericarp had 3.5 – 47.1 µg/g (Table III) (9, 19). The levels of the flavones in the tan plant sorghum varieties is on average an order of magnitude higher than values reported for common sources like celery (35). Thus the flavones in sorghum are likely to contribute significantly to health benefits of sorghum, particularly the tan plant varieties.

Apart from sorghum, a diverse array of flavones, including tricin, orientin, vitexin, among others, have also been identified in other cereal grains, though generally at low levels. Among the grains that contain significant levels of flavones are wheat, millet, oats, and fonio. In fact fonio (Digitaria exilis) was reported to contain relatively high levels of luteolin and apigenin (150 and 350 µg/g, respectively) (36). This is partly attributed to the relatively large proportion of pericarp (where most flavonoids are concentrated in grains) in the grain; fonio is a very small seeded grain with 1000 kernel weight of only 0.5 – 0.6 g, compared to 20 – 35 g typical for sorghum.

### Table III. Flavone and flavonone levels (µg/g) in sorghum grains of different plant secondary color and pericarp color

<table>
<thead>
<tr>
<th>Plant color</th>
<th>Pericarp color</th>
<th>Flavone levels (µg/g)</th>
<th>Flavanone levels (µg/g)</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tan</td>
<td>White</td>
<td>19.4</td>
<td>nd</td>
<td>(19)</td>
</tr>
<tr>
<td>Tan</td>
<td>Lemon yellow</td>
<td>268</td>
<td>1,428</td>
<td>(19)</td>
</tr>
<tr>
<td>Tan</td>
<td>Reddish/brown</td>
<td>60 – 386</td>
<td>8.1 – 48.4</td>
<td>(9, 19)</td>
</tr>
<tr>
<td>Purple/Red</td>
<td>Lemon yellow</td>
<td>24.8 – 47.1</td>
<td>694 – 1,780</td>
<td>(19)</td>
</tr>
<tr>
<td>Purple/Red</td>
<td>Reddish/brown</td>
<td>3.5 – 20.8</td>
<td>26.0 – 238</td>
<td>(19)</td>
</tr>
<tr>
<td>Purple</td>
<td>Black</td>
<td>36.2 – 40.9</td>
<td>trace</td>
<td>(9)</td>
</tr>
</tbody>
</table>

Values based on 12% moisture.

**Flavonols**

Flavonols share the same backbone structure with flavones, except for the hydroxyl group at C-3 of the flavonols. Thus the flavonols are 3-hydroxyflavonones (alternatively, flavones can be viewed as 3-deoxyflavonols, much like the relationship between anthocyanins and 3-deoxyanthocyanins) (Figure 5b). Various flavonols have been identified in cereal grains. However, these compounds are relatively rare in sorghum and have only been sparsely reported. Original report of flavonol in sorghum was by Nip and Burns (37), who identified kaempferol-3-rutinoside-7-glucuronide in a red pericarp sorghum. More recently quercetin 3,4′-dimethyl ether was identified in non-grain sorghum tissue.
(38). Again, the limited evidence of flavonols in sorghum suggests sorghum biosynthetic pathways favor the 3-deoxyflavonoids over the 3-hydroxyflavonoids (6).

![Flavone](image1.png) ![Flavonol](image2.png) ![Flavanone](image3.png) ![Flavan-4-ol](image4.png) ![Flavan-3-ol](image5.png)

**Figure 5.** Major classes of monomeric non-3-deoxyanthocyanin flavonoids found in sorghum. Flavones (a): Apigenin, $R_1 = H$, $R_2 = H$; luteolin $R_1 = OH$, $R_2 = H$; 7-O-methyl derivatives, $R_2 = CH_3$. Flavonols (b): Kaempferol, $R = H$; Quercetin, $R = OH$. Flavanones (c): Naringenin, $R = H$, Eriodictyol, $R = OH$; 5-O-glycosides and 7-O-methyl derivatives of these compounds are also found in sorghum. Flavan-4-ol (d): Apiforol, $R = H$; luteoforol, $R = OH$.

**Flavanones**

Flavanones have the basic 2,3-dihydroflavone structure, i.e., differ from flavones by the lack of a double bond between C-2 and C-3 (Fig 5c), they thus
have one chiral center at the C-2 position. Flavanones are widely distributed in nature since they are key intermediates in flavonoid biosynthetic pathway. In food plants, they are however most readily associated with citrus as the major dietary source. For example, naringenin in grapefruit has been widely studied for its health as well as potentially adverse pharmacological effects. Among grains, sorghum accumulates relatively high levels of flavanones (Table III). The major flavanones identified in sorghum are primarily eriodictyol and naringenin as well as their glycosides (39–41). We have also detected 7-O-methyl derivative of naringenin, as well as other unknown flavanones in some sorghum varieties.

Like other flavonoids, levels of flavanones in sorghum is highly influenced by genetics, especially genes coding for pericarp color, with apparently minimal influence of secondary plant color (9, 19). Lemon yellow sorghums have the highest levels of flavanones regardless of plant color (Table III). In fact the levels of flavanones reported in these sorghum varieties (694 – 1,780 µg/g) are much higher than those reported for citrus (including grapefruit) fruits which range between 400 – 600 µg/g on a fresh weight basis ((19, 42). Thus the flavanones in the lemon yellow sorghums are likely to contribute significantly to any health benefits derived from consuming these grains. However, as of yet, no studies have reported bioactive properties of the lemon yellow sorghum cultivars.

Even though secondary plant color does not affect overall levels of flavanones in sorghum, it seems to affect the type of flavones present (9). These authors reported that the predominant flavanones in the purple plant sorghums was naringenin and its derivatives, accounting for up to 80% of the total flavanones; while eriodictyol and its derivatives were more significant in the tan-plant sorghum varieties. The precise mechanisms by which genetics control the accumulation of the flavanones in sorghum and the specific genes involved need to be investigate further. Since the lemon yellow sorghums typically contain very low 3-deoxyanthocyanin levels, it is possible that silenced downstream genes in the flavonoid pathway lead to the unusual accumulation of the flavanones. Likewise, increased accumulation of 3-deoxyanthocyanins (as seen in black sorghum) leads to negligible levels of flavanones in sorghum (Table III). The potential health implications of these sorghums as well as possibility to use their bran as a concentrated source of the flavanones should be investigated. For example, the flavanones in sorghum bran fractions are 4 – 6 times the levels found in the whole grain (19).

**Flavan-4-ols**

Flavan-4-ols are flavone-derived alcohols (Figure 5d). In sorghums, flavan-4-ol compounds are synthesized from flavanones, naringenin and eriodictyol, and are considered precursors of 3-deoxyanthocyanins (43, 44). They include luteofor (leocoluteolinidin) and apifor (leucoapigeninidin) and are believed to play an important role in mold resistance in sorghum (45, 46). These compounds are common monomeric units of condensed tannins in sorghum, and can exist in significant quantities in some varieties. For example, Bate-Smith and Rasper (47) reported up to 900 µg/g luteofor in sorghum grain. In fact initial reports suggested that most sorghum tannins are polymers
of luteoforol (proluteolinidin) and apiforol (proapigenininidin) as opposed to the typical procyanidins found in other plants (47, 48). This may be true for some varieties; however, later reports suggest a greater diversity in sorghum proanthocyanidins. In general sorghums with tan plant color have lower levels of flava-4-ols than the purple/red plant sorghums.

Flavan-3-ols

Flavan-3-ols (also sometimes simply referred to as flavanol) are a subclass of flavonoids that contain 2-phenyl-3,4-dihydro-2H-chromen-3-ol skeleton (Figure 5e). Flavan-3-ols are widely distributed in fruits, vegetables, and other food plants like tea and cocoa beans, and are the main building blocks for procyanidins (a form of condensed tannins). The most common flavan-3-ol monomers found in food plants include catechin, epicatechin, and epigallocatechin. In cereal grains, barley and sorghum are the major commodities that contain catechin and epicatechin, along with their polymers. In sorghum these compounds are mostly found in their condensed polymeric form (49).

Proanthocyanidins (Condensed Tannins)

The group of flavonoid compounds most readily associated with sorghum are traditionally the condensed tannins. Adverse effect of tannins on nutrient digestibility in animal feeding rations is well documented, and has been the subject of countless studies. Breeding efforts to select sorghums with no condensed tannins (to improve feed efficiency) over the years have been largely successful, resulting in very limited production of tannin sorghums at present. Condensed tannins (also referred to as proanthocyanidins because they release anthocyanidins on acid hydrolysis) are polymeric forms of mainly flavan-3-ols and flavan-4-ols and their derivatives (Figure 6). The degree of polymerization ranges from 2 to more than 10. The polymerization typically occurs via C4→C8 interflavan bonds, referred as a B-type linkage (Figure 6a – c). Additional ether bonds between C2→C7 can exist in some of the tannin polymers producing what is referred to as the A-type linkage (Figure 6d). These A-type tannins have been associated with health benefits in cranberries (50).

In cereal grains, condensed tannins are rarely accumulated in the seed, and have only been reported in sorghum, barely, and millets. In sorghum, the tannins initially identified were the 3-deoxyproanthocyanidins, proluteolinidin (14, 47) and proapigenininidin (48). However, a greater diversity in composition of sorghum tannin polymer has been documented over the years, with catechin, epicatechin, gallicatechin, epigallocatechin, eriodictyol, along with glycosides of some of these monomers as chain extenders or terminators (41, 51, 52). However, whether sorghum genetics is associated with the accumulation of specific structural conformation of the tannins is not known.

In sorghum, the tannins typically accumulate in a discrete layer known as a pigmented testa, which is sandwiched between the endocarp and the aleurone layer parts of the pericarp. The genetics of tannin accumulation in sorghum is well documented. Generally sorghums without the pigmented testa do not contain...
tannins; the presence of the pigmented testa is controlled by $B_1B_2$ genes (5). Dominance at both loci is required for a pigmented testa to be present; for example, $B_1b_2b_2$ or $b_1b_1B_2-$ will not have the pigmented testa, and thus will not contain any tannins. In addition, sorghum is classified into three types depending on the levels of tannin present in the grain; type I sorghums do not contain any tannins (lack a pigmented testa). Type II and III sorghums both have the $B_1B_2$ genes, and thus contain tannins. However, type III sorghums also contain a dominant $S_-$ gene (spreader gene), which makes the pericarp color phenotypically brown (53) and levels of extractable tannins (up to 68 mg/g reported) (54) much higher than in type II sorghum (typically 0.2 – 8.0 mg/g). Thus tannin-containing sorghums are commonly referred to as ‘brown’ sorghum.

Figure 6. Some proanthocyanidin (condensed tannin) structure identified in sorghum. For dimers, $n = 0$, otherwise $n = 1 – 10$. Glc; glucose.
Tannins in general are structurally very diverse, and derived their name from their ability to tan leather, which basically involves the tannins binding to collagen protein. Tannins are also well known for their interaction with salivary proteins which causes the feeling of astringency commonly experienced when consuming wine, tea, fruit skin, etc. The interaction between tannins and proteins is believed to be an important part of plant defense against herbivores, bird depredation, among others. In fact, tannin sorghums are commonly referred to as “bird resistant” sorghum, since birds will generally not consume them if other food options are available. Sorghum tannin binding with proteins is highly selective and involves both hydrogen bonding and hydrophobic interactions. Proteins that are rich in proline with an open structure and relatively large molecular weights bind preferentially with tannins (55, 56). Proline residues disrupt the protein α-helix structure, leading to an open conformation with carbonyl and amide groups extending outwards, thus maximizing opportunity for hydrogen bonding and hydrophobic interactions.

The tannin-protein binding is usually very strong, and such complexes are largely indigestible. In addition tannins are good metal chelators, and thus can significantly reduce bioavailability of multivalent minerals like iron and zinc. A large body of evidence accumulated over the years has demonstrated significant effect of feeding tannin sorghums on suppressed weight gain by monogastric animals. The effects are attributable to not only nutrient binding, but also inhibition of enzymes as well as intestinal brush-border bound amino acid transporters. However, the tannin interaction with various dietary components is today largely considered a plus, particularly in regards to human health, in terms of reducing dietary caloric intake, increasing antioxidant status in the gastrointestinal tract, among others (54, 57).

**Part II: Health Properties**

**Introduction**

Increased whole grain consumption is universally recognized as one of the best ways to improve human health. Whole grain consumption is associated with reduced risk of various chronic diseases, including cancer, heart disease, obesity, diabetes, among others. Even though dietary fiber, as well as important vitamins and minerals in whole grain contribute to some of the benefits, it is clear from numerous studies that the overall benefits cannot be explained by these components alone. It is obvious that the flavonoids and other phenolic antioxidants concentrated in cereal brans are important contributors to health benefits of whole grain. As mentioned previously, whole grains are in general low in phenolic compounds, especially when compared to the so called ‘super fruits’. However, grains are much more widely consumed on a consistent basis, and in higher quantities than fruits and other high antioxidant products, and are thus likely to produce tangible benefits from their antioxidants. Additionally, grains are high in bound phenolic compounds that likely contribute more of the antioxidant-related benefits in vivo than can be measured in vitro.
The levels of flavonoids and other phenolic compounds in sorghum, on the other hand, are much higher than most grains and rival those in the ‘super fruits’. This makes it likely that sorghum will contribute to the health benefits associated with whole grain antioxidants at much reduced levels of consumption. Data on specific health benefits of sorghum flavonoids is still relatively limited, but growing with the expanding consumer interest in whole grains and ‘ancient grains’. We summarize some of the data documenting the potential benefits of various flavonoids found in sorghum and their implications in the following sections.

Antioxidant-Related Mechanisms

Given the central role oxidative stress plays in chronic disease development, most investigations of health benefits of various foods commodities invariably begin with antioxidant assays. For a long time, key dietary vitamins, A, C and E have been known to share free radical scavenging as one of the mechanisms by which they protect humans from disease. The recognition that most fruits and vegetables that were associated with health benefits also contain high levels of phenolic compounds that had antioxidant capacity similar to or more powerful than that of the vitamins, led to the overall shift of perspective from the vitamins and minerals as the primary source of health benefits associated with fruit and vegetable consumption. Even though most flavonoids are generally poorly absorbed in the small intestine (estimated at about 5% on average), the overall consumption is relatively high compared to that of the antioxidant vitamins. For example, is estimated that daily consumption of flavonoids in the typical American diet is about 210 mg/day (58); with people who consume tea regularly, the levels can be as high as 800 mg/day, according the Pauline Institute of Oregon State University. By comparison, average daily intake of vitamins C is less than 80 mg/day, while that of vitamin E is less than 10 mg/day.

In most cereal grains, reported antioxidant activity is generally low when compared to fruits and vegetables; this is partly due to their low levels of flavonoids and extractable phenolics in general. As stated earlier, most phenolics in cereals are strongly bound to cell wall material and cannot be extracted in typical organic solvents used in antioxidant assays. However, sorghum stands out among cereal grains owing to its high levels of flavonoids (for example, Tables I and III). In fact, levels of flavonoids in sorghum exceed those reported for high dietary phenolic sources like fruits and tea. For example, tea, which contributes the bulk of dietary flavonoids in the American diet, contains 130 – 300 mg flavonoids (mostly in the form of catechins) per 235 mL cup (59). By comparison, type III sorghums contain 28 – > 60 mg catechin equivalents (CE)/g proanthocyanidins, with levels in bran exceeding 150 mg CE/g (20). With a reported approximate 50% loss during cookie processing (49), cookies made with 5 g type III sorghum bran each could supply more than 350 mg CE/cookie. In addition, the sorghum also contains significant levels of other flavonoids, like 3-deoxyanthocyanins, flavones, etc, that would contribute significantly to overall flavonoid intake.

It is thus not surprising that sorghum has been shown to have much higher free radical scavenging activity when compared to other cereals grains, or even
fruits and vegetables (54). For example pigmented sorghum varieties show 10 – > 20 times the antioxidant activity of red wheat (20). The antioxidant activity in various sorghum varieties is strongly correlated with their phenol and flavonoid content (7). Among all varieties of sorghum, tannin sorghums have the strongest antioxidant capacity; this is attributed to the generally higher free radical scavenging power of tannins relative to simple flavonoids (20, 57). Condensed tannins have more hydroxyl groups in close proximity to each other and are thus more effective at quenching peroxyl radicals than simple phenolics, and are not capable of acting as pro-oxidants via redox cycling (which is sometimes the case for simple phenolics) (57). These authors, also proposed that even though tannins are largely non-absorbable due to the large molecular weight and strong complex formation with macromolecules, they likely serve an important role as free radical sinks in the gastrointestinal tracts, thus sparing other dietary antioxidants, in addition to potentially protecting the gastrointestinal epithelial cells. The same could be logically said for the bound phenolic compounds in cereal brans (which form the majority of grain phenolics). They likely contribute more to the antioxidant properties of whole grain in the gastrointestinal tract than assumed.

Apart from direct free radical scavenging, various bioactive compounds, including some phenolics, are capable of stimulating synthesis of various endogenous antioxidant and detoxifying enzymes, e.g., glutathione reductase, quinone oxidoreductase, among others. However, such properties are not directly related to antioxidant activities of phenolic compounds as such, but more to their specific structural conformation. For example, recent evidence demonstrates that O-methyl substituted 3-deoxyanthocyanidins in black sorghum induce the phase II enzymes, quinone oxidoreductase in murine hepatoma cell model in vitro (Figure 7), which suggests ability to enhance phase II detoxifying enzymes in humans (12, 60). Such specific activity was not observed for non-methoxylated 3-deoxyanthocyanidins or other flavonoid groups in sorghum, and has not been reported for other cereals. Enhance activity of the phase II enzymes has been linked to chemoprevention, among other benefits. This implies that specialty sorghum varieties that accumulate the specific active molecules could be selected for targeted health applications.

Another common effect of many flavonoid antioxidants is anti-inflammatory activity. Chronic inflammation, which is directly related to oxidative stress, is believed to be a common pathway to various chronic diseases. Thus ability of bioactive compounds to keep inflammation in check is considered one of the most important predictors of health promoting potential. Emerging data suggests that sorghum flavonoids, and maybe yet unidentified compounds in sorghum, are especially potent anti-inflammatory agents. For example, bran extracts from various sorghum varieties, including tannin sorghum, and non-tannin sorghum with red, black and white pericarp inhibited the activity of hyaluronidase; over-expression of hyaluronidase can lead to chronic inflammatory conditions responses in macrophages and dendritic cells (62). Brans from tannin and black sorghum also reduced the release of cytokines after LPS (lipopolysaccharide) irritation in vitro, and reduced infection induced by TPA (12-O-tetradecanoylphorbol acetate) in rats (63). The anti-inflammatory effect correlated with phenolic content and antioxidant capacities of the brans.
Interestingly, wheat and rice brans did not show anti-inflammatory properties in each of the two studies. This suggests that the unique flavonoids in sorghum may be driving the observations. Another study (64) demonstrated that white sorghum bran extract suppressed IgE production by U266 cells, whereas wheat bran extract did not; the authors were unable to identify the compound(s) involved. Additional evidence indicates that sorghum pigments extracted from sorghum stem eliminated the oxidative stress in rat brain induced by cyclophosphamide which may suggest protective effect from oxidative stress-related neurodegenerative diseases (65).

![Figure 7](image)

**Figure 7.** NAD(P)H:quinone oxidoreductase specific inducer capacity of apigeninidin and its methoxylated derivative found in black sorghum compared to synthetic dimethoxyapigeninidin. Hepa1c1c7 murine hepatoma cell lines were used for the assay. Cells (10,000/well) were induced with extracts for 24 h before assay. Error bars represent ±sd from three separate experiments. *, P < 0.05; **, P < 0.001, compared to control (Bonferroni multiple-comparison test). Adapted from (11, 61).

Indirect evidence is also available for various flavonoids found in sorghum. Quercitin (66), naringenin (67), luteolin and apigenin (68), all found in sorghum, were shown to reduced the formation of prostanoids and leukotrienes during lipid peroxidation, by inhibiting eicosanoid generating enzymes, such as phospholipase A2.
Obesity, Glycemic Response, and Related Properties

Obesity (and related health problems) is undoubtedly one of the greatest health challenges facing mankind today. Until fairly recently, obesity was considered a problem exclusive to the developed world. However, cases of obesity and diabetes are increasingly inflicting developing world at rates that rival those in the developed world. For example, according to Organization for Economic Co-operation and Development (OECD), Mexico currently rivals the USA for the top spot of obesity incidences in the world. Unfortunately, the poorest of developing countries have not been spared either. It is not uncommon to find overweight and starvation coexisting side by side in many countries in Africa, for example. The fact that most weight loss/weight management strategies peddled over the past two decades by both credible and dubious entities have not produced much impact adds to the magnitude of the problem. It seems human beings have become victims of their own success; food production has improved by leaps and bounds in the past century, while technological advances and wealth accumulation mean that physical exertion is optional for an increasing segment of society.

Sorghum has been long known to have reduced feed efficiency relative to corn, particularly type III tannin sorghums. In particular, monogastric animals seem especially susceptible to type III tannin sorghum; reduced feed efficiency and weight gain by 10 – 50% have been reported when tannin sorghums (containing 25 – 35 mg CE/g) were fed to rabbits, pigs or chicken (69–71). In all these experiments, the feed efficiency of non-tannin and low tannin (probably type II) sorghums was identical to that of corn. Thus even though sorghum proteins (especially kaffirins) are known to cross-link during processing and slow digestibility of carbohydrates, it seems their influence on feed efficiency is modest at most.

The best documented mechanisms by which sorghum tannins reduce nutrient digestibility include binding of food proteins, and possibly carbohydrates, into non digestible complexes as previously explained. Such complexes become part of dietary fiber and may actually play an important role as antioxidants in the digestive tract (57), besides possibly modulating colon microflora. Besides directly binding to macro-nutrients, tannins can also bind to digestive enzymes including, amylases, proteases and lipases (72, 73), thus inhibiting their activity. Additional mechanism reported for sorghum tannins is via inhibition of intestinal brush-border bound amino acid transporters (74). Since these effects have not been reported for sorghums with the monomeric flavonoids, it is apparent that the polymeric tannins are the ones involved. There is no direct evidence for tannin sorghum consumption and glycemic properties or nutrient digestibility in humans. However, anecdotal evidence cited by Awika and Rooney (54) indicates that some cultures in Africa prefer tannin sorghums due to their long satiety effect, likely related to slowed nutrient digestibility by tannins.

Given the important role sorghum plays as a staple in many developing countries, strategies to increase its productivity and food use in these regions should be encouraged. As the developing world becomes increasingly aware of connection between diet and long term health, the use of traditional foods
like sorghum that had been abandoned to a large extent in urban middle class for the more ‘westernized’ diets will increase. However, providing scientific concrete evidence of whether and how sorghum consumption can contribute to weight management and incidences of diabetes will be crucial. In addition, such evidence would stimulate food use of sorghum in the rest of the world, especially as a component of calorie control dietary strategies. However, impact of tannin sorghums on micronutrient bioavailability, particularly zinc and iron, must be considered, especially in populations that are deficient in these nutrients.

Cancer

Positive correlation between whole grain consumption and cancer prevention is well documented; the evidence is especially strongest for gastrointestinal cancers. For example, Larsson et al 2005 (75) reported that colon cancer risk was reduced 33% in women who consumed 4.5 servings of whole grain per day compared to those who consumed less than 1.5 servings per day. Whole grain consumption was also associated with a 50% reduced risk for upper digestive tract (esophagus, oral cavity and pharynx) (76). Countless other studies support these findings. Whole grain contributes these benefits likely through a combination of factors; including antioxidant activity, particularly of the bran cell wall-bound phenolic compounds; soluble and insoluble dietary fiber which help regulate digestion as well as colon microbiota; and via phytoestrogenic properties contributed by lignans and other estrogenic compounds in whole grain.

Among cereal grains, sorghum has had the most striking evidence for its potential to benefit chemoprevention. Van Rensburg (77) reported reduced incidences esophageal cancer in various parts of the world (Africa and Asia) where sorghum consumption was high, whereas consumption of maize and wheat correlated with elevated incidences. The authors proposed that sorghum may act by mitigating the adverse effects of micronutrients deficiency that increased the risk of such cancer in these areas. An epidemiological study by Chen et al. (78) revealed similar findings for Sachxi Province, China. This study included 21 communities within the province over a period of 6 years and found that regions that consumed highest amounts of sorghum, and to a lesser extent millet, had 1.4–3.2 times lower mortality from esophageal cancer than areas that primarily consumed wheat or corn. The authors corrected for consumption of other foods like alcohol, tea, meats and vegetables. Growing incidences of squamous carcinoma of the esophagus among blacks in South Africa was attributed to the change in diet from sorghum to maize (79). Available evidence clearly suggests that there is something in sorghum that contributes to cancer prevention over and above what other grains may provide. It has been suggested that the apparent chemoprotective effects of sorghum is due to the fact that sorghum has much lower incidences of aflatoxin relative to, say corn. However, the fact that other grains like wheat which are also very low in aflatoxin don’t show similar benefits suggests that there are actually active components in sorghum that contribute the benefits.

Emerging evidence with cell culture models does demonstrate that there is indeed more to the unique sorghum flavonoids than mere speculation. Shih et al
(8) compared the two primary 3-deoxyanthocyanidins in sorghum, luteolinidin and apigeninidin, with their analog anthocyanidins, cyanidin and pelargonidin, respectively, for ability to inhibit cancer cell growth in vitro. They found that luteolinidin and apigeninidin were much more effective at reducing cancer cell proliferation than their analogs at all the concentration tested. For example, at 200 µM concentration, luteolinidin inhibited HL-60 leukemia and HepG2 liver cancer cell proliferation by approximately 93% and 47%, respectively (Table IV). At equivalent concentration, cyanidin inhibited the cells by 24% and 0%, respectively. Yang et al (12) reported relatively potent inhibitory effect of apigeninidin and luteolinidin and their various derivatives against HT-29 colon cancer proliferation, with many of the compounds inhibiting cell growth by 50% at below 50 µM. The authors reported that O-methyl substitution improved the inhibitory properties of the 3-deoxyanthocyanin compounds, with 5,7-dimethyl substituted forms being most active. They also reported in the same study that black sorghum pigment extract was much more potent at inhibiting the HT-29 cell proliferation than commercial red cabbage pigments; the sorghum extract inhibited 50% of HT-29 cell proliferation at less than 200 µg/mL, whereas red cabbage extract was ineffective even at 800 µg/mL.

**Table IV. In vitro inhibition of human leukemia (HL-60) and liver (HepG2) cancer cell proliferation by 3-deoxyanthocyanidins, luteolinidin and apigeninidin, compared to their anthocyanidin analogs, cyanidin and pelargonidin. Data transformed from Shih et al (8)**

<table>
<thead>
<tr>
<th>Cell line</th>
<th>Concentration</th>
<th>Luteolinidin</th>
<th>Cyanidin</th>
<th>Apigeninidin</th>
<th>Pelargonidin</th>
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<tr>
<td>HL-60</td>
<td>100 µM</td>
<td>55</td>
<td>85</td>
<td>70</td>
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<td></td>
<td>200 µM</td>
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<td>35</td>
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<td>HepG2</td>
<td>100 µM</td>
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<td>200 µM</td>
<td>53</td>
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</table>

Percent cancer cell proliferation relative to untreated control at two concentrations of the compounds shown.

Other evidence we have gathered indicate that tannin sorghums also show much higher potency than grape seed extract at inhibiting the HT-29 cancer cell growth (Figure 8). In fact Awika et al (60) reported that tannin sorghum extracts were more potent than black, red or white sorghum extracts at inhibiting HT-29 as well as OE33 esophageal adenocarcinoma cell proliferation. The tannin sorghums inhibited 50% of HT-29 and OE33 cell proliferation at 38 – 65 µg/mL. These values are much lower than those reported for most natural extracts (typically above 1000 µg/mL). The evidence clearly suggests that the sorghum compounds have much higher potential to suppress preformed cancer from rapid proliferation than their analogs found in other food sources, and may be relevant for dietary applications at low use levels.
Figure 8. Comparative inhibition of in vitro HT-29 colon cancer cell proliferation by sorghum pigment and tannin extracts, and commercial red cabbage and grape seed extracts. Cells (3000/well) were incubated with extracts for 48 hrs before assay, MTT method was used. Error bars represent ± sd based on three separate experiments; unpublished data.

Cardiovascular Health

Whole grain consumption is well known to correlate with reduced incidences of cardiovascular disease (CVD). For example, a large 10 year study (80) found that the level of whole grain intake inversely correlated with the risk for coronary heart disease among women, with the highest level of intake associated with 50% reduction of risk. The authors also concluded that the level of CVD risk reduction could not be fully explained by the contribution of whole grain dietary fiber, folate, vitamin B-6, and vitamin E, which implies other components may be important. Whole grain is a good source of dietary fiber, minerals, vitamins, antioxidant and other beneficial compounds. Pinpointing the active components of whole grain that benefit cardiovascular health is thus a difficult task. However, some data suggests that the bran components and not the germ (which is rich in minerals and vitamins) contribute to the benefits of whole grain consumption (81). The authors reported that whole grain consumption in general reduced coronary heart disease risk by 18% among men, whereas added bran in diet reduced the risk by 30%; added germ did not produce any benefit. We are not aware of any studies that compare benefit of different sources of whole grain on CVD risk.

Uncontrolled free radical challenge and chronic inflammation in the body are known important causes of CVD. It is thus not surprising that many compounds the act as antioxidants and anti-inflammatory agents also tend to benefit cardiovascular health. For example, low density lipoprotein (LDL) oxidation
may lead to artery wall damage and eventually atherosclerosis by increasing inflammation in the blood vessel wall. Dietary antioxidants are believed to play an important role in keeping LDL oxidation in check. Numerous epidemiological studies have shown that consumption of products high in flavonoids, like red wine and tea correlate with reduced risk of CVD (82, 83). These effects are largely attributed to the tannins and other polyphenols in these food products. Even though there is no epidemiological data on tannin sorghum consumption and CVD risk in humans, it is likely that sorghum tannins may have similar benefits. In addition, the monomeric 3-deoxyflavonoids are also likely to contribute to cardiovascular health, given their demonstrated high antioxidant and anti-inflammatory properties. With the growing risk for obesity and CVD in the regions where sorghum consumption is significant, a study in this regard is highly warranted.

Concluding Remarks

Sorghum contains a diverse array of flavonoid compounds, most of which are not typically found in other cereal grains. The high levels of compounds like 3-deoxyanthocyanins, pro(3-deoxy)anthocyanidins, flavones, and flavanones in certain sorghum varieties is especially of interest both from a commercial and health perspective. Given that the types and levels of the flavonoids in sorghum are controlled by a set of well documented genes; it is possible to breed for sorghum varieties that accumulate specific compounds at levels that can be exploited for targeted food/health applications to produce desired benefits. For example, the levels of 3-deoxyanthocyanin pigments in black sorghum bran as well as purples sorghum plant glumes and other plant tissues are high enough to warrant commercial interest as natural food colorants. The fact that these sorghum pigments are relatively stable compared to other natural colorants is an added advantage. The high levels of flavanones in lemon yellow sorghum varieties, and proanthocyanidins in type III sorghum could provide an economical way to obtain these well known bioactive compounds for nutritional applications.

Evidence suggests that the unusual compounds in sorghum and the high levels at which they are present in the grain produce specific health benefits that are not observed for other grains like corn, rice, or wheat. The epidemiological evidence that indicate sorghum consumption may be more chemoprotective, especially against gastrointestinal cancer, is an interesting example that warrants additional investigations. The laboratory evidence based on cell and animal models seem to support the epidemiological evidence for higher efficacy of sorghum relative to other grains. The fact that sorghum 3-deoxyanthocyanins are more cytotoxic to cancer cells; that O-methylated 3-deoxyanthocyanins are powerful phase II detoxifying enzyme inducers; that sorghum brans elicit anti-inflammatory response not observed for other cereal brans in vitro and in vivo; that sorghum phenolic extracts are more powerful antioxidants than other grains, or fruits and vegetables, among other, all warrant a closer look at sorghum as a commodity that can significantly contribute to chronic disease prevention.

Important questions remain as to how sorghum components contribute to the various observed superior benefits. Whether it is simply related to
better membrane permeability of, say, the 3-deoxyanthocyanins relative to anthocyanins, or specific structural conformations that fit better with target cell receptors, etc, are unknown. The biochemical basis for these observations must be unraveled. Other broader questions will need to be addressed: What levels of sorghum consumption would produce desired benefits in humans? (This would be particularly interesting from a long term disease prevention perspective, rather than short term therapeutic-type studies.) How would the different sorghum varieties with varying flavonoid composition benefit specific health outcomes? How can food processing be optimized to maximize the beneficial effects of sorghum flavonoids?

The fact that sorghum is currently mostly consumed in the developing world has been somewhat a setback in terms of stimulating widespread research into the specific health benefits of the grain, especially in relation to chronic disease. However, with the growing number of ‘Western’ health problems like obesity and cardiovascular disease in developing countries, an opportunity to gain better insight into sorghum consumption and disease prevention is upon us. An additional question of interest is whether sorghum flavonoids could contribute to strengthening of the immune system; this would be particularly important given the scale of HIV in Africa, which is also the largest sorghum consuming region.

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